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ISAH

ANIMAL HEALTH & WELFARE
BIOSECURITY
ENVIRONMENTAL PROTECTION
SUSTAINABILITY OF ANIMAL HUSBANDRY

XIII INTERNATIONAL CONGRESS IN ANIMAL HYGIENE ISAH–2007

June 17–21, 2007, Tartu, Estonia

ANIMAL HEALTH, ANIMAL WELFARE AND BIOSECURITY

PROCEEDINGS VOLUME II

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"ANIMAL HEALTH, ANIMAL WELFARE AND BIOSECURITY"

PROCEEDINGS VOLUME 2

Editor: A. ALAND

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International Society for Animal Hygiene
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Tartu 2007

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Dept. of Animal Health and Environment
Institute Of Veterinary Medicine and Animal Sciences
Estonian University of Life Sciences
Kreutzwaldi 62
51014 Tartu, Estonia
Phone: +372 7 313 221
Fax: +372 7 313 706
E-mail: Andres.Aland@emu.ee

Congress Secretariat ISAH-2007
Frens Conference Services
Toompuiestee 30
10149 Tallinn, Estonia
Phone +372 697 9290
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FOREWORD

On behalf of both the Organising Committee and the Scientific Committee, I am pleased to welcome you in Tartu, Estonia, to participate at the XIII International Congress of the International Society for Animal Hygiene (ISAH).

The ISAH (www.isah-soc.org) was founded in 1970 and has today members from 48 countries throughout the world. ISAH can be considered as a group of scientists contributing to efficient, sustainable animal farming with healthy animals, providing wholesome food in a sound environment.

Veterinarians and non-veterinary academic scientists (animal science, agricultural economics, engineers, microbiologists, public health professionals, epidemiologists etc., etc) and respective professionals in animal husbandry, who work and/or do research and education in the field of animal hygiene, can apply for a membership of ISAH, and are most welcome to attend ISAH congresses.

The first ISAH congress was held in Budapest in 1973. The last ISAH main congress took place in Warsaw, Poland in 2005 and the last in-between symposium in Saint-Malo, France in 2004.

Starting from Warsaw congress in 2005, the ISAH, considering the need for a more flexible and frequent exchange of scientific and practical knowledge, organizes its congresses every second year.

The present, XIII ISAH congress in Tartu, Estonia, in June 17–21, 2007 is organised under the device **"Animal health, animal welfare and biosecurity"**.

The scientific programme, trying to follow the scope of the ISAH and receive the feedback from modern animal husbandry and food production, concentrates with more profoundness on the following subjects: interaction between the environment and health and welfare of individual animal and herds; managing animal health in large dairy units; ensuring animal welfare during transportation and slaughter; economical implications considering animals' health; possibilities of precision livestock farming in maintaining good health and welfare of animals; measures for prevention the development and spread of diseases and pathogens in animals including those posing risk to human health (zoonoses); food safety relevant infections and contaminations such as residues in food derived from animals; influence of the animal production on the environment and public health.

The Proceedings from the XIII ISAH Congress are herewith presented. The papers on lectures from invited speakers, oral and poster presentations from 11 parallel sessions are included in this excellent compilation. In general, the printed contribution to the ISAH-2007 congress illustrates clearly the broad scientific field of the ISAH and related to it activities.

I hereby would like to express my most sincere gratitude in the address of ISAH-2007 organising and scientific committees. Special thanks go to Frens Conference Services for their excellent organizational and technical contribution and to AS Triip for their outstanding printing job of these proceedings. We also appreciate different companies and organisations for their considerable financial support which gave us the opportunity to keep the registration fees affordable.

Finally, we thank all participants, contributors, chairpersons, organisational and technical assistants for your considerable efforts – you made the ISAH-2007 in Tartu real success.

We wish you all interesting and pleasant congress and enjoyable stay in Tartu.

A. Aland
Editor
Chairman of the ISAH-2007 Organising Committee

CONTENTS

POSTER PRESENTATIONS

POSTER PRESENTATIONS

A3 – IMPACT OF ENVIRONMENT ON POULTRY BEHAVIOUR, HEALTH AND PERFORMANCE

ORAL PRESENTATIONS

POSTER PRESENTATIONS

Tuesday, June 19, 2007

PLENARY LECTURES

PARALLEL SESSIONS B1; B2, C; D

B1 – ANIMAL HEALTH – ECONOMICAL ASPECTS IN ANIMAL HUSBANDRY

ORAL PRESENTATIONS

POSTER PRESENTATIONS

POSTER PRESENTATIONS

C – PRECISION LIVESTOCK FARMING – HEALTH AND WELFARE ASPECTS

ORAL PRESENTATIONS

POSTER PRESENTATIONS

D – FREE COMMUNICATIONS

ORAL PRESENTATIONS

Wednesday, June 20, 2007

PLENARY LECTURES

PARALLEL SESSIONS E1, E2, F, G

E1 – MICROBIAL RISK – OCCURRENCES IN SPECIES AND HUSBANDRY SYSTEMS AND RISKS OF SPREADING

ORAL PRESENTATIONS

POSTER PRESENTATIONS

POSTER PRESENTATIONS

G – ANIMAL TRANSPORT, EUTHANASIA AND SLAUGHTER – CAN GOOD WELFARE BE PROVIDED?

ORAL PRESENTATIONS

POSTER PRESENTATIONS

Tuesday, June 19, 2007

PARALLEL SESSIONS B1; B2, C, D

C –
PRECISION LIVESTOCK FARMING – HEALTH AND WELFARE ASPECTS

ORAL PRESENTATIONS

RISK ASSESSMENT CHALLENGES IN THE FIELD OF ANIMAL WELFARE

Candiani D., Ribò O., Afonso A., Aiassa E., Correia S., De Massis F., Pujols J. and Serratosa J.

Animal Health and Welfare (AHAW) Unit, European Food Safety Authority (EFSA), Largo N. Palli, 5/A, 43100 Parma, Italy.

SUMMARY

EFSA provides scientific advice regarding risks associated with food by using the Risk Assessment (RA) approach. The AHAW Panel of EFSA provides advice on risk factors related to animal diseases and welfare. The EFSA Scientific Colloquium on Food Producing Animals (2005) concluded that no standard methodology on RA for Animal Welfare (AW) exist yet. This paper presents the different RA approaches developed by EFSA, when assessing the risks associated with AW, from the Calves' Welfare Scientific Opinion until the current approaches on Pig and Fish welfare. These constitute the basis for the future standardization of a RA methodology for AW.

Keywords: animal welfare, risk assessment, hazard identification, hazard characterisation, exposure assessment, risk characterisation, food producing animals.

INTRODUCTION AND OBJECTIVES

The mission of the European Food Safety Authority (EFSA) is to provide scientific and technical advice for the Community's legislation and policies in all fields which have a direct or indirect impact on food and feed safety, including animal health and welfare (http://www.efsa.europa.eu). The Animal Health and Animal Welfare (AHAW) Panel of EFSA provides advice on specific risk factors related to animal diseases and welfare of food producing animals, including fish.

EFSA provides independent assessment on all matters within its remit by using a Risk Assessment (RA) approach. RA is the process of evaluating the likelihood and severity of an adverse event occurring to humans, animals or to the environment following exposure under defined conditions to a specific hazard. Guidelines for the conduct of RA have been developed to assess the risk of animal disease import (OIE, 2004a and b) and the risk of microbiological hazards in food (Codex Alimentarius, 1999).

In order to discuss the state of the art regarding the RA in food producing animals, a Scientific Colloquium was organized by EFSA on December 2005 and held in Parma (EFSA, 2006c). One of the main conclusions from the colloquium was that no specific standardized RA methodology exists in the field of the Animal Welfare (AW) and that it would be worthwhile to set up a working group to further investigate on these methodologies.

The main difficulty in AW seems to be the clear description of adverse effects and distinction from causal hazards which are crucial for the characterisation of the risk. In addition, it has to be taken into consideration that hazards and adverse events may be different depending on species, breed, age, physiological status and production system.

Since 2004, the Animal Health and Animal Welfare (AHAW) Panel adopted several Scientific Opinions on AW dealing, among others, with laboratory animals, stunning and killing methods, piglet castration and animal transport. In 2006, two Scientific Opinions on the welfare of intensively kept calves (EFSA, 2006a) and the health and welfare risks of the import of captive birds inside EU (EFSA, 2006b) were adopted. A new approach on the RA methodology was tentatively initiated. At present, new scientific opinions dealing with pig welfare, fish welfare and dairy cows' welfare are under development where this RA approach on animal welfare is being improved.

The aim of this paper is to present the different approaches developed by EFSA's Working Groups, when assessing the risks associated with AW in food producing animals, starting from the Calves' Welfare Scientific Opinion (EFSA, 2006a) until the current ongoing scientific opinions on Pig and Fish welfare.

METHODS AND RESULTS

As previously referred no specific methodology exists for the development of the RA in AW. Therefore, both OIE and Codex methodologies were adapted to the AW field in order to develop a step by step scientific RA. The first RA approach was attempted in the Scientific Opinion on Calves' Welfare (EFSA, 2006a). The second approach on the health and welfare risk of the import of captive birds solved some of the gaps from the previous Calves RA approach. Finally, the ongoing risk assessments on pig and fish welfare improve the first attempt on developing a RA approach on AW. The different steps followed on the development of the different scientific opinions are explained:

1. Hazard identification

Hazard is defined as a production factor affecting AW while the risk is a function of the probability of a negative effect on the animals and the severity of that effect (adapted from OIE, 2004a). Hazard identification consists in the recognition of the biological, chemical and physical agents able of causing adverse effects on AW (adapted from WHO, 1999). The first step for achieving hazard identification was to identify the animal's needs. Such animal needs, which must be fulfilled at farm level (e.g. need to obtain resources, receive stimuli or express particular behaviours) were related to one of the three main sources of risk: nutrition, housing and management. This allowed identifying the production factors which constitute the hazards. Examples of needs, related hazards and adverse effects on the animals are reported in Table 1. This first step was commonly followed on the different AW scientific opinions (calves, captive birds, pig and fish welfare).

Table 1. Examples of hazards related to animal needs with related adverse effects

Need	Hazards	Adverse effect
Nutrition: to drink, to thermoregulate,…	• Difficult access to water • Insufficient feed • Too low milk T° • …	• Thirst • Hunger • Stress, anxiety • …
Housing: to rest, to exercise,…	• Sliding floors • Inappropriate ventilation • …	• Lameness • Pain, malaise • …
Management: To avoid fear, to have proper social interactions,…	• Staff without experience • Mixing of unfamiliar animals • …	• Stereotypes • Fear • Stress • …

2. Hazard characterisation

Hazard characterisation is the qualitative and/or quantitative evaluation of the nature of the adverse effects associated with the hazard (adapted from WHO, 1999). In the scientific opinion on Calf Welfare, the impact of the various hazards on the individual animal was evaluated and referred to as slight, adverse, moderate, serious and very serious according to the severity of the hazard effect on the animal.

During the development of the scientific opinion on Captive birds (EFSA, 2006b), the effect on the individual animal was correlated to the duration of the hazard: it was therefore introduced the duration parameter. The difference in the hazard characterisation estimation between the Calf and the Captive birds' scientific opinions is presented on Table 2.

The ongoing RA on the welfare aspects of different husbandry systems for farmed pig and for farmed fish introduce the parameter of hazard magnitude, including both duration (relative to the whole animal life time) and severity (from negligible to critical) of the adverse effect, and the parameter of likelihood of the occurring of the adverse effect (Table 3).

Severity has been divided in critical (when it is fatal); severe (explicit pain, malaise, fear or frustration may occur); moderate (some pain, stress, fear or anxiety reactions); limited (minor pain and malaise) and negligible (no pain, fear or frustration occur).

The uncertainty of the scientific evidence on the likelihood was also introduced in respect to the principle of transparency. Uncertainty is low when solid and complete data are available (peer-review published data); medium when no complete data are available or authors' conclusions vary from one to other; and high when scarce or no data are available or for rather evidence provided in unpublished reports (Table 3).

Table 2. Hazard characterisation in the Calves and the Captive birds' scientific opinions

Calf Welfare	**Captive Birds**	
	Hazard characterisation	
Hazard characterisation	**Severity**	**Duration**
Very Serious	Critical	Short (0.5 h)
Serious	Severe	Medium (12 h)
Moderate	Moderate	Long (24–48 h)
Adverse	Limited	Very long (> 48 h)
Slight	Negligible	

Table 3. Hazard Characterisation – Pig Welfare and Fish Welfare

Hazard characterisation					
Hazard description	**Adverse effect description**	**Magnitude**		**Likelihood**	**Uncertainty**
		severity	**Duration**		
		Critical	0–100%	High	Low
		Severe		Moderately high	Medium
		Moderate		Moderately low	High
		Limited		Low	
		Negligible		Negligible	

3. Exposure assessment

Exposure assessment is the qualitative and/or quantitative evaluation of the exposure to the production factors which may cause an adverse effect (adapted from WHO, 1999). In the Calves' Welfare and Captive birds' RA approaches, the exposure to the hazard was determined in terms of likelihood and intensity of the exposure from the animal.
The parameters of duration and uncertainty, relative to the exposure assessment, were also introduced in the Pig Welfare and Farmed fish Welfare RA approaches, as shown in Table 4.

Table 4. Exposure Assessment – Pig Welfare and Fish Welfare

Exposure assessment			
Intensity	**Duration**	**Likelihood**	**Uncertainty**
Critical	0–100%	High	
Severe		Moderately high	Low
Moderate		Moderately low	Medium
Limited		Low	High
Negligible		Negligible	

4. Risk characterisation

Risk characterisation is the estimation of the probability of occurrence and severity of the adverse effects in a given population following the exposure to a specific hazard (adapted from WHO, 1999). Risk characterisation gives the risk managers information on the specific situation of the animal in relation to its basic needs. In the Calf Welfare and Captive birds (EFSA, 2006 a, b) scientific opinions, the overall risk on animal welfare was estimated by integrating the hazard characterisation and the exposure assessment into risk estimations (major, minor or negligible risk; Table 5). A similar approach, including the evaluation of the severity/intensity and the duration in both hazard characterisation and exposure assessment, will be followed for the risk estimation of the Pig and Fish Welfare Scientific Opinions.

Table 5. Risk characterisation – Calf Welfare and Captive Birds' scientific opinions, 2006

Risk characterisation		**Exposure assessment**				
Hazard characterisation		**Very rare**	**Rare**	**Moderately frequent**	**Frequent**	**Very frequent**
	Slight adverse effect	Negligible risk	Negligible risk	Negligible risk	Negligible risk	Negligible risk
	Adverse effect	Negligible risk	Negligible risk	Negligible risk	Negligible risk	Minor risk
	Moderately serious	Negligible risk	Negligible risk	Minor risk	Minor risk	Minor risk
	Serious	Negligible risk	Negligible risk	Minor risk	Minor risk	Minor risk
	Very serious	Negligible risk	Minor risk	Minor risk	Minor risk	Major risk

CONCLUSIONS

The RA process has several benefits. The major advantage is transparency as scientific evidence is provided through the data used, the risk pathways and assumptions are defined and the RA approach is described. RA can support the prioritization of areas for intervention (risk management) and give information on further data needs (recommendations for future research).

As previously described, different RA approaches have been followed for the development of the scientific opinions in the field of AW. The Scientific Colloquium of 2005 concluded that a standardized methodology for RA in AW does not exist at the moment. As a consequence, EFSA is launching a self-mandate on the establishment of general guidelines and working methodology for RA in AW issues. The work has already started with the set up of the necessary basic information, which includes the definition of the scientifically justified main issues to be considered and a list of key researchers and centres of excellence working in AW and RA related with AW (at EU and not EU level).

The next step for the development of the RA Guidelines in AW will be the set up of different Working Groups, in relation to the main animal species and AW issues to be considered.

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PLAYING GAMES FOR THE FUTURE: A METHOD FOR CONSTRUCTION AND EVALUATION OF SCENARIOS FOR SUSTAINABLE ANIMAL PRODUCTION

Gunnarsson, S.[1], Sonesson, U.[2], Nybrant, T.[3], Stern, S.[4] and Öborn, I.[5]

[1] Department of Animal Health and Environment, Swedish University of Agricultural Sciences (SLU), P.O.Box 234, SE-532 23 Skara, Sweden; [2] Swedish Institute for Food and Biotechnology (SIK), Göteborg, Sweden; [3] Department of Biometrics and Technolog;, [4] Department of Animal Breeding and Genetics; [5] Department of Soil Sciences, SLU, Uppsala, Sweden

SUMMARY

Sustainability within agriculture is a complex issue. In this study we have worked with a forecasting scenario technique. A methodology for working with scenarios for future agricultural production systems was developed. The scenarios can then be evaluated both quantitatively, e.g. economy and life cycle analysis (LCA), and qualitatively, e.g. animal health and welfare. The method has been used on pig production, beef production, dairy production and the production of food potatoes.

Keywords: sustainable agriculture; animal health, animal welfare, scenario method

INTRODUCTION

The Swedish research program FOOD 21 are working with sustainable food production in many aspects; plant nutrient management, animal welfare and production, consumer research, farmer interaction, systems analysis modelling and food quality (FOOD 21, 2004). In order to communicate the massive amount of results from the program a synthesis work was initiated.

Sustainability is doubtless a complex subject with many aspects. In agriculture, sustainability contains a large portion of ecological issues, if the environment is damaged you can not sustain your production since it relays on natural systems (Tilman et al., 2002). The social as well as economic aspects of sustainability are also important since agriculture is important in rural areas. An often used term when discussing sustainability is the "triple bottom line", referring to the three main aspects of sustainability; ecological, economical and social. This means that true sustainable requires that all these three aspects are taken into account. For sustainable agriculture there is not a triple bottom line, but a "quadruple bottom line", since there is also an agriculture-specific aspect of animal husbandry; animals are used for producing food, which brings in ethical considerations on how we treat our animals. In agriculture there are several conflicting goals between the four sustainability aspects. Examples of conflicts are: economic efficiency is increased by using less space for the animals which involves reduced animal welfare. Another example is that in order to promote a natural behaviour for animals deep litter bedding should be used which probably increases the emissions of ammonia. Moreover, agriculture is a complex business, it consists of biological production in an environment that is neither easy measurable

nor controllably (as compared to industrial production). Rigby and Caceres (2001) propose that agriculture can be sustainable on three levels; field, farm and society level, considering that sustainability means managed in a way that enables the system to continue its activities into the future. Following that definition, agriculture can be sustainable on field level even if the farm is not sustainable, and it is also possible that a farm can be sustainable in a non-sustainable society. Hence they conclude that it is very important to state the level of sustainability, when discussing agricultural sustainability.

There are articles presenting actual production systems and their sustainability. Sundrum (2001) presented a review of several sustainability aspects (even though he did not mention the word sustainability) in organic livestock production, as environmental protection and product quality but focused on animal welfare. In contrast to Sundrum (2001), who discussed sustainability aspect on farm level, de Wit and co-workers (1995) article approached a societal level of farm sustainability. This means that they also wanted aspects as food shortages and equity of food supply to be included in the criteria for sustainability. One feasible way of elaborating sustainability issues generally, but also within agriculture, is to use scenario methodology. Scenarios originally were used for military purposes and when the method entered the civil society it was in economy and management, an example from management literature is Schoemaker (1995). The objective of this study was to develop a method to design scenarios for future agricultural systems that can be used for different productions, as pig, milk and arable farming.

MATERIAL AND METHODS

We chose a back casting scenario approach since the purpose was to develop a method for constructing scenarios that are more sustainable than today's system, not to present different outcomes of varying policies or technologies. We were also working with a definition of sustainability as an ability to fulfil certain criteria, as described by Hansen and Jones (1996), and sustainability on farm level, according to Rigby & Caceres (2001). The core principles in the method are transparency and structure. This means that when the method has been used for constructing scenarios, all assumptions are explicit, all choices made are clear and conflicting goals are identified. A reader should be able to use the process scheme and follow the scenario construction all the way from the values used for stating the goals through the process, understanding all choices made and be able to judge how relevant the choices are. The structure in the method is important to help the people involved to think of the production system unbiased of how the system looks today.

A very important background for the development of the method is the assumption that the most efficient way of incorporating experts from different fields of agricultural research as well as authorities and business is to present concrete descriptions of scenarios on meetings. This will initiate discussions about the relevance of the choices made in designing the scenarios and possibilities to develop the scenarios based on these "expert meetings". Hence the process described below is of an iterative nature.

1. Define and describe the value base that will guide the work.
2. Define systems boundaries and describe the system.
3. Define all relevant sustainability parameters (called "focus parameters"). This is a list of parameters relevant for the studied system. In our work we mainly used the list of

"sustainability goals" defined by FOOD 21 (FOOD 21, 2004), but it is obvious that other definitions of sustainability can be used.

4. Describe all sub systems that make up the entire system.
5. Formulate "focus scenarios". A focus scenario is a scenario where the system is optimised for just one focus parameter. To formulate a scenario is to describe how all functions are solved principally and technically, e.g. describing how the animal feed is composed and delivered to the animal or what tillage methods are used. These focus scenarios are rather extreme, taking only one aspect into consideration for every sub system. The principal solution for a sub system is described, e.g. "the manure must be removed quickly from the house". This "principal concept" is then transferred to an "implementation concept" which is a technical description how the principal concept can be achieved. It is not necessary that there are technical solutions ready on the market but technical concepts that could be developed based on today's knowledge. At this stage is it not absolutely important to find the best solution, the scenarios developed here will be refined later in the process.
6. Identifying conflicting goals. Conflicting goals are solutions for a sub system that are chosen to optimise one focus parameter that will obstruct the optimisation of another goal.
7. Describe goal visions. A goal vision is a description of what sustainability aspects that are most important, as decreasing emissions, save scarce resources or working environment and animal welfare.
8. Describe goal vision scenarios. One new scenario, goal vision scenario, per goal vision is described. A goal vision scenario is a description of how the system should look if the focus parameters belonging to that goal vision are optimised.

   In earlier steps (point 5) two levels of solutions, concepts, are described, principal and implementation concepts. These two levels can be regarded as two time horizons. Principal concepts are not definite in time but can work as guides to where we should strive. The implementation concept on the other hand, is solutions that are possible to implement in a rather short time frame. Hence, by combining the principal concept we get a scenario that are more far away and by combining the implementation concept we get a scenario that are feasible in the short term. Conclusively two goal vision scenarios for each goal vision is designed, one principal and one implementation. On the latter it is possible to make rather detailed quantification regarding both economical and environmental impact, but for the former the accuracy of quantifications is lower.

   When goal vision scenarios for the first system are ready, the same procedure is applied on the next system that is part of the total production system under study. With the goal vision scenarios for the system needed ready, next part of the process begins which is to combine the goal vision scenarios.
9. Design goal vision scenarios for the total system.
10. Meeting with experts.
11. Modification of the scenarios.
12. Evaluation of the goal vision scenarios. Life Cycle Assessment combined with farm modelling was used for quantifying the environmental effects. For an economic analysis it is necessary to make assumption about agricultural policies and prices of input resources. The qualitative evaluation was based on literature review and panel discussions with groups of experts/stakeholders.

RESULTS AND DISCUSSION

The method described in this article is developed within a Swedish research project, but the approach is not limited to Swedish or even European agriculture. The stepwise method where single sustainability issues are dealt with one at a time is general for agriculture. The definition of "Goal visions" can be very different from the one relevant for Swedish agriculture but still the method works and produce the same transparency. The structured working process, describing focus scenarios for the system where just one sustainability parameter is optimised, facilitates free thinking since it allows the working group to disregard all other aspects which leaves room for new ideas. The feasibility of these new ideas will later in the process be tested and perhaps form part of a new solution in a scenario. The focus scenarios are also valuable since they facilitate "traceability" in the process; it is possible for the receiver of the results to identify the whole process from the entire scenario back to every single choice on every sub-system and function.

The main aim with developing the method described was to find a method of developing scenarios in a more transparent and structured way. Since the scenarios are both rather concrete and logically constructed, they can be very valuable when the issue of sustainable agriculture is discussed. Such scenarios, and quantified results from them, can work as platforms for discussions between different stakeholders since they provide a mutual and concrete picture of different perspectives of sustainability. The explicit descriptions of goal conflicts that is a result of the method is very useful to realise where the conflicts between different interest lies. Our method deals with two time frames, principal long term scenarios and implementation short term scenarios, but it builds on the same goal visions, i.e. sustainability goals. At the same time as concrete and detailed descriptions are needed, the method also must entail discussions on a rather high systems level; otherwise the scenarios will not fulfil the aim of presenting examples of more sustainable systems. By using the method, a wide range of systems levels, from definitions of sustainability to descriptions of housing for animals, are considered in a logical way. In Figure 1 schematic picture of the different systems level for the steps in the method is presented.

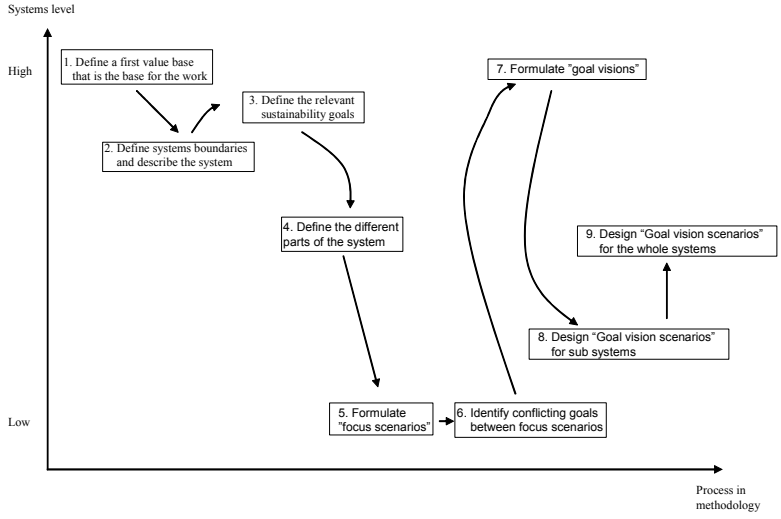

Figure 1. Schematic presentation of the different systems level considered in the steps in the methodology

The feasibility of using the method for other production systems, as industrial production, is not analysed, as it is not within the scope of this study. One very specific asset of agriculture is the strong interdependency between many parts in the system, as soil, plant, animals, technology and humans. These interrelationships makes it very difficult to analyse parts of the system alone since choices made for one part often heavily affects other parts. Another difference compared to industrial production is the large range of products delivered from the system, and where the production of each one often is connected to several others, e.g. crops in a crop rotation or animals fed with crops from the fields. There are certain limitations of the method. One limitation is that the method is used on farm level; e.g. how is milk production best performed. The matter of the sustainability of the food system as a whole is not addressed. This aspect includes what products should be produced and in what amounts. The question of where different products are best produced is also omitted. Other aspects not covered are "margin effects", i.e. what will happen if the need for arable land increases or decreases. A second disadvantage is that even if the aim is to synthesise scientific knowledge into more comprehensible pictures of more sustainable systems, there is a risk that important information can be neglected. There is no absolute methodological mechanism that guarantees the completeness of the scenarios.

The method has been tested on Swedish pig production (Stern et al., 2005), dairy production (Gunnarsson et al., 2005) and beef production (Kumm et al., 2005) and the experiences from that work are promising; the process facilitated free thinking and it was possible to manage very different system level. The latter means that both a very concrete discussion about the systems can be achieved in the same study as a more hypothetical discussion about conflicts between sustainability goals in a long time perspective. But since the actual goal of agricultural production is to deliver raw material to either the food industry or directly to consumers via retail, an analysis of the whole chain up to consumption would be an important step. A final important conclusion is that the scenario work should be performed by a team composed of persons experienced within the field of study and some type of systems analysis. It is matter of finding generalists rather than specialists, when putting together the group (Lund et al. 2006).

CONCLUSIONS

The method presented herein offers a structured way of synthesising large amount of research knowledge into something comprehensible and practically understandable that can be used as a platform for further discussions about sustainable agriculture. The resulting scenarios can be subject to external assessment of all steps in the process. The method has been used on pig production, beef production, dairy production and the production of food potatoes. It offers a structured way of synthesising large amount of research into something comprehensible and practically understandable that can be used as a platform for further discussions about sustainable agriculture.

ACKNOWLEDGEMENT

All persons within companies and organisations that gave their perspectives and comments on the scenarios are also acknowledged. Furthermore, we thank Mistra, the Swedish foundation for strategic environmental research, which financially supported the research programme FOOD 21.

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CLAW PATHOLOGIES, DAIRY COW GAIT AND CLAW SIGNATURE

Nuffel, A.V.[1], Gyselinck, N.[2], Maertens, W.[1], Goolaerts, A.[2], Van Aert, M.[2], Opsomer G.[2], and Sonck, B.[1]

[1] Agricultural Engineering, Technology and Food, Institute for Agricultural and Fisheries Research, Merelbeke – Belgium; [2] Department of Obstetrics, Reproduction and Herd Health, Faculty of Veterinary Medicine, Ghent University, Ghent – Belgium; annelies.vannuffel@ilvo.vlaanderen.be

SUMMARY

In order to assess associations between claw pathologies, claw signature and cow gait, 24 dairy cows were examined during six weeks. The health of the claws was not extremely bad and only few cows had a gait score resulting in classification as lame. More detailed individual associations between claw pathologies, claw signature and cow gait will be presented at the conference. Results indicate that there is an association between gait scores and the duty cycles of different legs. Further research is needed to support these findings.

Keywords: dairy cow, lameness, gait, claw

OBJECTIVE

For dairy cows, claw disorders are amongst the most important health problems during their active life. Research shows that claw problems are responsible for 90% of the lameness cases on dairy farms (Weaver, 2000). It has furthermore been demonstrated that the early detection of the affected animals is of paramount importance to reduce pain suffering and to enhance the chance of healing. In this context, the search for associations between claw disorders and gait abnormalities has already had some attention but major difficulties due to the subjective nature of claw and gait scoring still remain. To examine the association between manifestly and subclinically present claw disorders on the one hand and cow gait parameters and claw signature on the other hand, the following experiment was carried out.

MATERIALS AND METHODS

Animals and experimental setup

Between 25th of October and 29th of November 2006, 24 lactating Holstein cows of mixed parity were examined at the Ghent University Research Farm (AgriVet). During these six weeks, three groups of eight cows were guided to the test arena after milking once a week. This test arena was situated in a nearby empty stable and consisted of three different experimental zones (fig. 1; C, D and E).

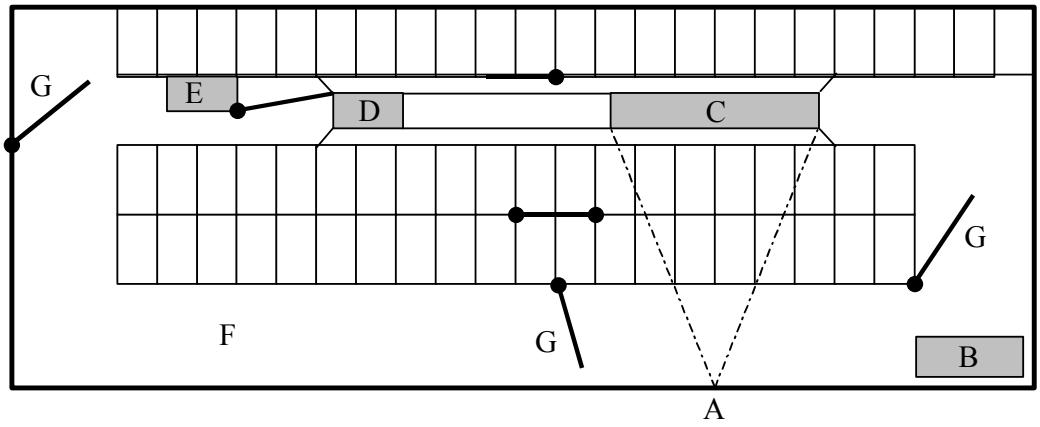

Figure 1. Scheme of test arena (30 x 11 m²) and experimental setup: (A) digital video camera, (B) cow balance, (C) walking corridor (6m), (D) cow box with pressure plate, (E) trimming chute, (F) waiting area, (G) separation fences. Cows circulate anti-clockwise.

To give the cows the opportunity to adjust to the new environment, they were guided several times through the experimental setup. Subsequently, each cow was tested individually according to the following protocol: First, cows were guided to walk through a six meter long corridor, on a recently roughened slatted concrete floor (fig 1;C) while their gait was filmed with a digital video-camera (fig. 1;A). Secondly, the cows were guided into a box of 1 * 2 m² (fig. 1;D), the hind legs were positioned on top of the RSscan® pressure mat and the pressure signature of the claws of both hind legs was recorded. Finally, the cows were guided into a trimming chute (fig. 1; E) to examine the claws of both hind legs for any kind of disorder. Photographic pictures of the claws were taken from several sides and the claw angle was measured using a goniometer (fig. 2). The cow's weight was measured in a cow balance (fig. 1;B) during week six.

Figure 2. Measurement of the claw angle with a goniometer

Claw Pathologies and claw angle

During this period of six weeks, the claws were weekly scored for the presence of specific lesions. Both hind legs were examined (first the right, then the left) in the trimming chute by the same veterinarian for the following clinical disorders: digital dermatitis (DD), interdigital dermatitis (ID), sole ulcer (SU), white line disease (WL), laminitis (LA), interdigital necrobacillosis (IN), interdigital skin hyperplasia (IH) and overgrowth of the sole horn (SH). To do so, the claws were cleaned with a small brush and superficial horn was gently scraped off to remove all the dirt and manure. In this way, superficial lesions could be visualized and identified using the Dutch identification system (GD Ltd., 2006). If present, lesions were scored based a four-point scale (score 0 = no visible lesions, score 3 = severe lesions). The relevancy of the claw lesions in relation to the lameness of dairy cows has been confirmed by Alban (1995); Manske (2002); Somers et al (2003) and Flower and Weary (2006). The claw angle of each hind claw (claw angle, CA) was measured using a goniometer (Fig. 2). As a claw angle of 45° is regarded as optimal, the following angle scores were used: 0: [43°–47°]; 1: [41°–42°, 48°–49°]; 2: [36°–40°, 50°–54°]; 3: [≤ 35°, ≥ 55°].

Dairy Cow Gait

Based on the video material of the cows walking through the six meter long corridor, five observers scored the gait of the cows according to the method described in Winckler and Willen (2001). Manual frame by frame analysis and identification of claw strike (claw contacting the ground at end of swing) and claw-off (claw leaving the ground at end of stance) events in relation to (longitudinal) position and time were performed on these images with ad hoc written software. Based on this data, the variables summarised in table 1 were estimated.

Claw Signature

The pressure between the claws and a Rsscan pressure mat (0.96 * 0.32 m², covered with a 5 mm rubber mat) was measured for 30 s. The pressure mat consists of a left and a right side, so the measurement was repeated with the left and the right side of the pressure mat exchanged. Frames within a steady state pressure distribution were selected for further processing.

RESULTS

Cow parameters

Mean weight of the cows was 621 ± 56 kg. Most cows (70%) were in their first lactation stage (day 28 to 366), the remaining cows had lactation stages of 2 (day 72 to 305), 4(day 96 to 362) or 5 (day 426 to 468).

Claw scores (pathologies and claw dimensions)

The medial and lateral claws of both hind hooves were examined. No presence of laminitis or interdigital necrobacillosis was detected at any of the claws. Only 4% of the claw lengths were normal, 92% of the claws were long (> 7 cm) but without curved dorsal wall. Finally, 4% of the claws were extremely long with most of them showing a curved dorsal wall. The claw scores

(relative frequency of pathologies and claw angle scores) are summarised in figure 3. No analyses are performed on the photographic pictures of the claws yet.

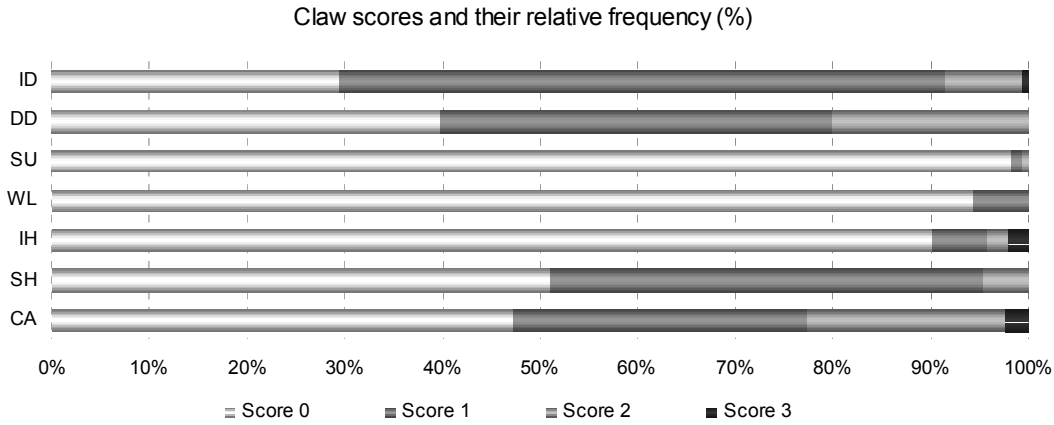

Figure 3. Relative frequency distribution of the scores of following claw disorders: interdigital dermatitis (ID), digital dermatitis (DD), sole ulcer (SU), white line disease (WL), interdigital skin hyperplasia (IH), overgrowth of the sole horn (SH) and claw angle (CA). No laminitis (LA) or interdigital necrobacillosis (IN) were detected and therefore they are not included in the figure.

Dairy Cow Gait

As the gait scoring based on the digital images was performed by 5 observers, an overall gait score was given to the cows by taking the modus of the 5 different scores. 89% was classified as sound (63% score 1 and 26% score 2). 11% of the assessed gaits was classified as lame with 8%, 2% and 1% for score 3, 4 and 5 respectively.

A set of basic cow gait variables is presented in table 1. An overall mean value (n = 156) and standard deviation of these variables is shown. Other variables determined from the manual frame by frame analysis on the digital images (e.g. on individual hooves, pairs of hooves, deducted variables, etc.) and their relation to the gait and claw scores still need to be investigated.

Table 1. Estimated cow gait variables and their description

Variable		Estimated value	
Stride time	Time between the sequential steps of one individual hoof	1.20 ± 0.23	s
Stance time	Time during one stride, at which the hoof is on the floor	0.77 ± 0.17	s
Swing time	Time during one stride, at which the hoof is in the air	0.43 ± 0.08	s
Duty cycle	Stance time relative to stride time	0.64 ± 0.03	%
Double support	Relative time, during walking, that the cow is on 2 hooves*	0.47 ± 0.10	%
Triple support	Relative time, during walking, that the cow is on 3 hooves*	0.55 ± 0.10	%
Stride length	Distance between the sequential steps of one individual hoof	1.56 ± 0.08	m
Tracking length	Distance between a front hoof and a subsequent hind hoof imprint	–0.01 ± 0.07	m
Speed	Stride length divided by stride time	1.34 ± 0.22	Ms^{-1}

* During a walking gait, the cow supports on 2 or 3 different hooves alternatingly.

Claw Signature

Due to a rather short measuring time (maximum 30 s) and some agitation of the cows due to the presence of flies, it was not possible to consider the number of times that a hoof was lifted as a useful variable. In between, the pressure distribution remained fairly constant over time. It seemed difficult to achieve a good "square stance" on the mat, so even more repetitions to account for this variation might have been desirable. The results of the claw signatures from the RSscan® pressure mat are not yet available.

CONCLUSIONS

The claw health of the cows in our study seemed to be not extremely bad. Only the prevalence of ID and DD was rather high (94% and 67% of the cows had at least one claw with a non-zero score for one of these pathologies respectively). Only 13% and 3% of the cows suffered from IH and SU respectively, often resulting in severe lameness. The prevalence of WL was 19%. As the cows needed to be trimmed in the near future, the high presence of cows with some overgrown sole horn (98%) or with long claws (97%) could be expected. 47% of the claws had claw angles near 45% (scored 0), but only 17% of the cows had 4 of these claws.

The mean speed of our cows was 1.35 ms^{-1} which is higher than the speed found in literature (0,8 ms^{-1} – Philips and Morris, 2000; 1,1 ms^{-1} – Flower and Weary, 2005; 1,0 ms^{-1} – Telezhenko, 2005). In 4 cows, significant differences could be found between duty cycles of different legs which imply that the gait of these cows was irregular. The gait scores of 2 of these cows were indeed 3 and 4 but the gait scores of the other cows were 1 and 2. Further research is needed to explain these results. Average double and triple support time was 47% and 55% respectively.

As Winckler and Willen (2001) only consider cows with gait score 3, 4 or 5 as lame, only 11% of the cows in our experiment were classified as lame. Literature however shows that the prevalence of lameness in dairy herds is around 26% (Barkema, 1994), or between 4% and 25% (Booth, 2004). These results show that the claw health of the cows in our study was rather good. Individual associations between gait scores, hoof pathologies will be determined and presented during the conference.

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REMOTE MONITORING AND ANALYSIS OF INDOOR TEMPERATURE, DAIRY COWS' HEALTH AND PRODUCTIVITY IN LARGE LOOSE-HOUSING COWSHEDS BY AN AUTOMATIC NETWORK SYSTEM

Poikalainen, V.[1], Veermäe, I.[1], Praks, J.[1], Aland, A.[1], Kaart, T.[1], Kokin, E.[1], Pastell, M.[2], Ahokas, J.[2] and Hautala, M.[2]

[1] Estonian University of Life Sciences, Kreutzwaldi 64, 51014 Tartu, Estonia;
[2] Department of Agrotechnology, P.O.Box 28, FI-00014 University of Helsinki, Finland

SUMMARY

Some elements of health and welfare monitoring integrated automatic system have been developed and tested at large uninsulated loose housing cowsheds in Estonia. The paper describes preliminary results of these tests, in particular the analysis of relationships between animals' health, microclimate and productivity. It is possible to work out integral automatic health and welfare control system for dairy cattle as a part of precision livestock farming framework.

Keywords: dairy cow, precision livestock farming, microclimate, welfare, automation, health, lameness

OBJECTIVE

Objective of the study was to create and test an automatic network-connected data acquisition system for:

1) monitoring microclimate, health status and productivity of dairy cows in large uninsulated cowsheds;
2) determining relationships between these parameters.

METHODS

The outlines of automatic monitoring system of animal welfare parameters and it's testing in large farm

The main information sources at the dairy farm for animal welfare assessment are:

1. data received by the controllers of milking parlour or automated milking system central control unit (Alpro Windows, STRANGKO) – milk yield, milk quality, health status, and so on;
2. measurement results of inner climate parameters (temperature, humidity, illumination, gas content of the air) of the animal sheds and milking parlour;
3. corresponding weather data;
4. observation results of animal behaviour in the milking parlour and sheds;
5. measurements of weight changes of the animals;
6. measurements of leg load variations depending on the health status;

7. breathing frequency and heart rhythms measurements data;
8. body temperature measurements data of the animals;
9. health status assessment based on the observations, and so on.

The accessibility of the data depends mainly on the possibilities of measurements at the given farm and in case of complex solution the existence of local measurement system is needed. The measurement data is accessible both by the farm personnel and animal welfare researchers.

That is achieved by connecting all farm computers, including milking parlour central control server, into local network (LAN). Computers responsible for additional measurements compose local research network (RLAN). The server in RLAN, usually computer with MS Windows Server 2003 operation system, does the administration of both networks. Measurement devices and data-loggers are connected to the network through Ethernet-switches. As some of the systems are wireless, corresponding WiFi-routers are also needed.

For remote control, measurements and collected data transfer from different farms to animals' welfare researchers, every farm's LAN has to have an access to the Internet. The most suitable way for that is establishing persistent connection to some Internet service provider by the telephone line with ADSL-modem.

To access local network and Internet, research network server is equipped with two network cards – one for connection with local network switch and the other for connection with ADSL-modem. Server should be configured as router and remote access server.

For more secure data transfer from research network to remote users, including researchers in Estonian University of Life Sciences, Virtual Private Connection (VPN) should be used.

Another possibility to access research network server is Remote Desktop service, but in this case there should be active Transactions Server (in farm or in University local network) that issues licenses to Remote Desktop users.

The third way is to use Virtual Network Connection (Real VNC) software that in case of farm network enables remote researcher to use the resources of the RLAN server and to connect through it to other local computers without interrupting the work of local users (contrary to Remote Desktop).

VPN, Remote Desktop and VNC connections are needed only for farm's local network administration and measurements control. To monitor the results of the measurements and to copy data files to workstations in EULS network it is more convenient to use Web Server Services of RLAN server, creating farm's Internet-homepage with appropriate design.

The measurement devices at the farm may be grouped by their connectivity to the computers:

1. Devices that are controlled and from which data is received through local computer network. In our case that were data-logger WebDAQ/100 with Web-server functionality and video-cameras NC1000-W10, also with Web-servers. In addition to that Web-cameras are wireless.
2. Devices that transmit measurement data by radio channel to the receiver, connected to LAN computer by RS-232 or USB interface. To that group belong automatic wireless weather-stations La Crosse WS2305 and MicroLog data-loggers with temperature, air humidity and illumination sensors for microclimate parameters measurements.
3. Devices that are connected directly to LAN computer by RS-232 or USB interface. The typical device belonging to this group is data-logger Spyder that may be used for measurements of signals coming from dairy-cows leg load sensors (kvasipiezoelectric mat, tensoresistive scales).

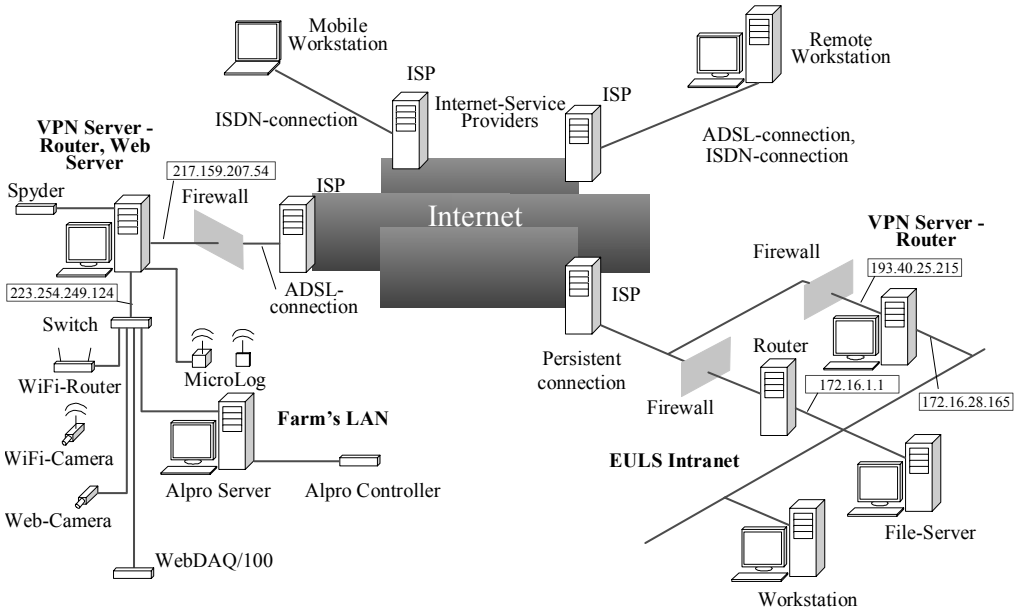

Figure 1. Farm's local network and Internet connectivity configuration

Figure 1 shows the configuration of farm's LAN, Internet-connection of this network and possibilities to access it from the EULS network and remote mobile workstations.

In case of such data-acquisition system structure all measurements results and data in milking parlour control system database are accessible through the farm's LAN and also to remote users in EULS network or elsewhere. To ensure appropriate level of security system servers should be equipped with firewall and virus protection software.

The first research network was created in summer of 2005 at AS Tartu Agro Vorbuse farm, where the milking parlour control system (DeLaval Alpro) database and backup files were copied regularly (once per day) to RLAN server. In 2006 automatic wireless weather station, video cameras and data-loggers for temperature, air relative humidity and illumination level measurements in sheds and milking parlour were added to the system. Measurements results and all other data are available at the farm's Internet homepage.

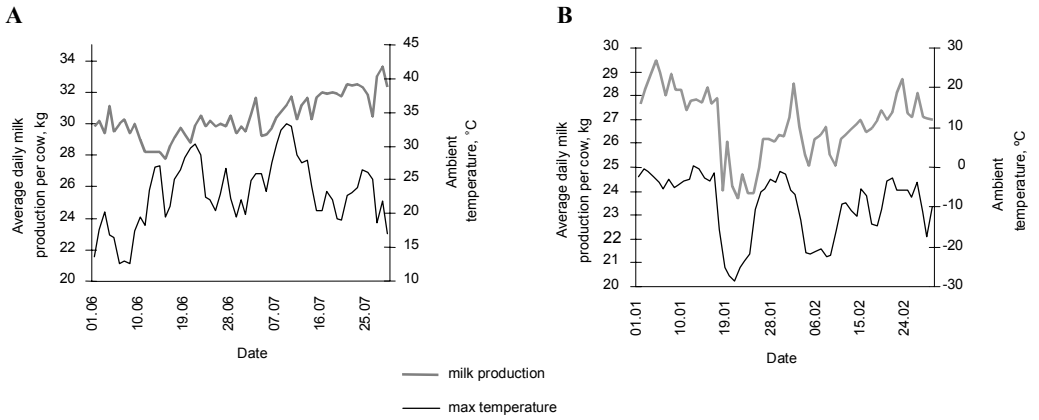

Figure 2. Relationships between ambient extreme temperatures and milk production per cow in summer (a) and winter (b)

At Pae Farmer farm and Torma POÜ farm automatic wireless weather stations and data-loggers for temperature, air relative humidity and illumination level measurements in sheds and milking parlours were installed.

At AS Estonia Kõrtsi farm the same measurement devices are used. In addition to that in December 2005 and January 2006 observations and video recordings of animals' behaviour during the process of customisation were made. The data received is not entirely analysed yet.

RESULTS

There were two extremely cold periods in January and February of 2006, when outdoor temperature dropped as low as –28.6 ºC. Cold periods lasted about a week each. In summer maximum temperature reached 33.3 ºC. Relationship between extreme outdoor temperatures and daily milk production per cow are given in Figure 2.

It appeared that during extremely cold period average daily milk production per cow dropped about 4 kg and remained low for a couple of days after the temperature had risen. The lower the outdoor temperature the bigger the decrease in milk production was. The same but in smaller scale appeared during hot period. This indicated the existence of thermal stress during these periods.

To study relationship between milk production and diseases of cows, milk production decrease of 20% was set as alarm level. The occurrence of diseases and corresponding number of low milk production alarms before diagnosis of disease are given in Table 1. The most frequent diseases were dyspepsia, mastitis, metabolic diseases and uterine infection. Corresponding decrease in milk yield was more evident in winter period and appeared in about 41% of cows with mastitis, 18% of cows with dyspepsia, 35% of cows with metabolic diseases and 11% of cows with uterine infection.

Table 1. Diseases and low milk production alarms in winter and summer

Disease	January-February			June-July		
	No of cases	No of alarms up to 6 days before	%	No of cases	No of alarms up to 6 days before	%
Abortion	1	1	100			
Abscess	2	1	50			
Dyspepsia	67	12	18	40	0	0
Enteritis	9	0	0	1	1	100
Exhaustion				16	6	38
Intoxication				1	0	0
Leg diseases	15	5	33	23	7	30
Mastitis	71	29	41	82	15	18
Metabolic diseases	54	19	35	12	3	25
Peritonitis				1	0	0
Respiratory diseases	21	0	0			
Rumen atony	6	5	83	2	1	50
Tetany				1	1	100
Trauma	1	0	0	8	1	13
Udder trauma	4	1	25	1	0	0
Uterine inflammation	45	5	11	42	3	7

It's evident that an association exists between climate conditions, productivity and cows' health. Based on the automatically registered climate and production data the changes preceding the disease can be recorded. Compared to the climate and production data corresponding to the healthy cows the relative risks and odds ratios as measures of the degree of association can be evaluated and their statistical significance tested. As the previous studies are showing the generalized linear models with logistic link function are suitable tools to model multifactor disease incidences. In future the changes in climate and production should be incorporated into a single complex model including also their interactions and possible confounding factors like cows' age and/or feeding. The adjusted relative risks calculated from the model coefficients express more precise and unbiased associations between climate conditions, productivity and disease incidences.

CONCLUSION

Automatic computer network systems can be used for monitoring of environment, health and production data of cows. The Internet access enables to analyse and evaluate relationships between these data remotely.

ACKNOWLEDGEMENTS

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INTERPLANT INFORMATION MODEL FOR PIG FATTENER IN FOOD CHAINS

Schütz, V. and Petersen, B.

University of Bonn, Institute of Animal Science, Preventive Health Management Group, Katzenburgweg 7–9, D-53115 Bonn; E-Mail: vschuetz@uni-bonn.de

SUMMARY

Extended documentation and self-control obligations also challenge the Farm-level primary production and cause additional work. In order to appropriate information for health and quality management a continuous inter-enterprise data flow and information processing is needed. The article shows how a consistent flow and processing of information can be guaranteed for owners of fattening pigs, by an online based documentation system, supported by analysis of information requirements. In connection with an interplant Data Warehouse the presented model envisions a joint use of information systems for fatteners and their services like veterinarians and advisor services.

Keywords: interplant information system, health and quality management, meat chain information, advisor service

INTRODUCTION

Legal requirements, especially by the EU-hygiene package (178/2002; 852, 853, 854, 882/2004 and 183/2005), as well as quality assurance systems from the private sector (DIN ISO 9000, QS, IKB, etc.), allege documentation and self-control obligations on producers of the food chain. Nevertheless, independently created isolated soft- and hardware solutions allow no optimum use of already digitized data for the quality and health management. Timely management of running processes avoids mistakes at the earliest on all production stages of breeding to trade (PETERSEN et al., 2002). If you look at the tasks of farmers and their advisor services as an element of a socio-technical closed loop system, the person takes the function of the regulator of a control path (PETERSEN, 1985). The area of responsibility the human adopts is controlled on the basis of descriptive, comparative and predicting processes. In pork based production chains the necessary categories of information are to be obtained by interlinking of the independent operating data systems which assure prompt and continuous data flow to the timely management of biological, technical and organizational processes.

MATERIAL AND METHODS

A five-stage model was applied by the integration of an on-line-based documentation system onto an interplant system of information and communication which takes into consideration organizational, technical and professional aspects. The model was modified by SCHULZE

ALTHOFF (2006) and includes the following stages whereas the first two stages are for planning and the following three for modelling and implementation:

1. Analysis of the actual and target information needs and ambition stage from pig fattener, advisor services, feed advisor services and veterinarians
2. Analysis of potential user and user groups and interplant unit of organisation
3. Development of the model, implementation and further developments
4. Reproduction of necessarily data inputs
5. Analysis of the interplant data

The central element here was to evaluate the information needs of different user groups. The data concerning actual and target information needs were collected by expert interviews with system provider, technical and veterinary advisers. For the questioning of 400 fatteners a standardised questionnaire was used. The questionnaire referred to the present state of the documentation and information systems in the stage of fattening as well as the needs and future arrangement of interplant systems. During the questioning period all together 187 questionnaires were send back and 183 were taken in account. In context to advancement of existing structures of information in service organisations, persons responsible in cattle utilization cooperatives, feed manufacturers and veterinary associations gave information. Involved were:

- *Service organizations of the animal mediation* from which it is demanded to deliver homogeny piglets within the agreed time in a defined quality, the same demands on the fatteners in regards to the pigs for the slaughter process.
- *Feed suppliers* who besides the advisor services are obliged to deliver feed in verified quality, given composition and in time.
- *Organisation of advisor services* which require in particular team counselling tailored information exchange between advisors and fatteners (PETERSEN et al., 1999).
- *Authorities and controlling institutions* which secure mutual obligations from the areas of quality assurance, animal, environment and consumer protection.

A product analysis showed the potential of existing software solutions for farmers. The developed model is currently tested upon practical feasibility and validation with the support of two system partners and one farm cooperative.

RESULTS

Demands of livestock owner

The evaluation shows that at present 64% of the interviewee rely on handwritten data documentation, 36% apply self-made software solutions, for example Excel, or rather special software fitted to their needs. Because level programs fulfil legal requirements only partially and therefore applying such methods in quality and health management is limited (PETERSEN et al., 2007). 83% of the interviewees fulfil legally prescribed documentation requirements as for example continuance accounting, by means of paper documentation. The entire daily documentation effort: 43% of the questioned masts estimate max. 5 minutes, 48% require 5–10 minutes and the remaining 9% require more than 10 minutes. With regard to the frequency of the documentation 28% of the interviewees document daily. 35% devote themselves 2–3 times a week to this task, 37% once a week or more seldom. Against the trend of documentation 75% of

the questioned fattener used computer-supported feeding systems, again 83% of the systems are isolated systems. Future demand of interplant information is estimated as very important in the area of feeding, preventive health management and evaluation of carcass. Traceability of the commodity flow as well as binding to existing systems is regarded as important. 44% of the questioned fatteners support a networking of data from offshore and post stored steps of pork production chains, under the condition that these are compatible with existing systems and important interplant information is made available. The decision concerning the way prospective supply of information from the fattening farm will be handled, half of the interviewees see the information handled via e-mail and an online system, the other half persists furthermore on paper or by verbal means.

Demands on service providers

The mentioned experts indicate to extend their offer to specific farm analysis and intercompany comparison in the future. Further more the questioning of the experts proved the following need for action: conception of new evaluation possibilities as well as optimization of the data exchange between the Farm-level operations and own organization; technical realization of interplant information exchange and supply of up till now missing hard-, soft- and orgware; combination of the organizational and technical conversion drafts to the extension of the service offer and the data use. In all three areas the interviewees still see research demand and a considerable investment risk for own developments.

INTERPLANT INFORMATION MODEL

The pilot testing of the presented concept of an online based documentation system in interplant information management sees four user groups: Feed companies, breeding, fattening and slaughterhouses (figure 1).

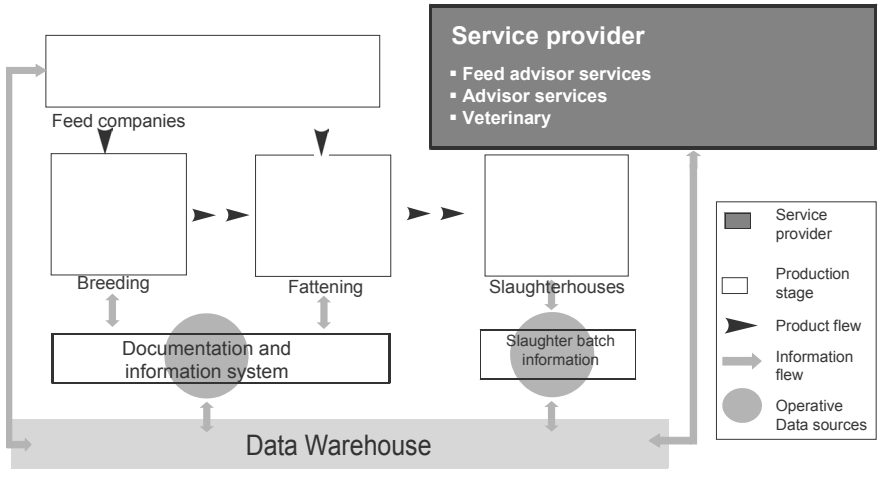

Figure 1. Interplant information model for farmers and their advisors services in meat chains

The model is closer explained only for the user groups pig fatteners and veterinarians. The farmer fulfils by input of specific information from incoming, out coming of the piglets medication and feeding the documentation and self-control obligations. Thereby it concerns a complementary documentation. Through the integration of data sources in the data warehouse digitized data from offshore and post stored steps are already illustrated by integration of surgical data sources in the documentation module. Thereby time-consuming and faulty double inputs are decreased. In addition to the PC version a transportable input device with scanning system or antenna supports the current input, at the same time required information can be called up directly with the working tasks in the stable. Using handwritten stable maps and transferring these onto a PC system is therefore omitted. The medicament document, application document and delivery document (this document is required by each delivery of medicaments in Germany by law) is for example an interplant record which is provided first in the practise software of the veterinarian. The veterinarian can electronically transmit the document by interfaces to the farmer. In the field of medication necessarily information can be documented online by the farmers as well as by the veterinarian. In addition the continuance book will be generated automatically. Data exchange before generating the medicament and application document makes it easier to specify the location and number of attended pigs. Additional entry masks make it possible for the veterinarian to fill in further information about the treatments. These can be used for specific analysis. If the farmer allows providing its veterinarian an insight into its data sets the veterinarian obtained the data concerted to their requirements. With the online platform veterinarian can document their visits, generate intercompany comparison, prepare visits and recognise aberration in an early stage. Entry masks for documentation in context of extended documentation or notification requirements e.g. during crises like food and mouth diseases support farmers and veterinarians by their business.

CONCLUSION

With the continuous spreading of the Internet on farms the need for on-line based documentation systems in connection with new DV-supported advisor services for farmer grows. The integration of such a communication potential in an interplant practised Data Warehouse system also assumes the reorganization of a number of service processes. Next development steps should be implement isolated software solutions into such interplant management system.

ACKNOWLEDGES

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ONLINE RECOGNITION AND LOCALISATION OF SICK PIG COUGH SOUNDS

Exadaktylos, V.[1], Silva, M.[2], Aerts, J.-M.[2], Taylor, C.J. [1], Ferrari, S.[3], Guarino, M.[3] and Berckmans, D.[2, 1]

[1] Engineering Department, Lancaster University, LA1 4YR Lancaster, United Kingdom;
[2] Department of Biosystems, Division M3-BIORES: Measure, Model & Manage Bioresponses, Catholic University of Leuven, Kasteelpark Arenberg 30, 3001 Heverlee, Belgium;
[3] Dipartimento di Scienze e Tecnologie Veterinarie per la Sicurezza Alimentare, Facoltà di Medicina Veterinaria, Università degli Studi di Milano, Via Celoria 10, 20133 Milano, Italy;
Corresponding author. Email: Daniel.Berckmans@biw.kuleuven.be

ABSTRACT

This research focuses on animal welfare and serves as an application to precision livestock farming. A sick cough recognition algorithm is combined with a localisation procedure to identify and localise sick animals (pigs in this work) in their housing environment. It is intended to provide continuous 24 hour health monitoring in pig houses and produce early warning in cases where treatment is needed. Early warning can provide the expert with valuable time to assess the importance of the alert and provide treatment only for the animals that pose a high risk.

Keywords: online sick cough recognition; online health monitoring; animal welfare

INTRODUCTION

Monitoring animal health in pig houses is vital for the sustainable management of a pig farm. Cough is a sudden air explosion in the airways followed by a characteristic sound (Korpáš *et al.*, 1996). Being one of the body's defence mechanisms against respiratory infections, it can be a sign of disorder or infection of the respiratory system. It has been identified as an index for over 100 diseases and experienced physicians can identify an infection based on the cough sound.

The importance of coughing as a means of prognosis does not refer only to humans, but also to animals. It has been shown that pig vocalisation is directly related to pain and classification of such sounds has been attempted (Marx *et al.*, 2003). It is also common practice by veterinarians to assess cough sounds in pig houses for diagnostic purposes. However, this approach cannot be used for continuous monitoring and early warning for infections in pig houses. In this regard, there have been attempts to identify the characteristics of coughing in animals (Moreaux *et al.*, 1999, Van Hirtum and Berckmans, 2001) and automatically identify cough sounds from field recordings (Van Hirtum and Berckmans, 2003, 2003a).

As the use of antibiotics has reached a level that cannot be accepted to guarantee the success of the drug in the future due to the increase of resistance in species, it is strongly recommended to decrease the use of it over all fields of applications. In this regard, apart from recognizing a sick animal sound, it is equally important to localise the sick animal in the compartment and immediately treat only animals in the pen of the animal that has been identified as sick.

In this paper, the aim is to investigate if the time domain characteristics of sound signals can be used to automatically classify sick coughs. Although the connection between the time domain characteristics of the sound and their biological meaning is not fully known, it is shown that they pose an attractive classifier for the case of sick pig coughs. For the localisation of the cough sounds, an algorithm based on the "Time Difference of Arrival" (TDOA) of the signal in 7 microphones is proposed. It is shown that this computationally efficient method provides acceptable accuracy levels for the specific application.

MATERIAL AND METHODS

Experimental data

Three data sets are used in this work. The first one is used to present the classification properties of the time domain characteristics, the second one for the evaluation of the localisation algorithm and the third one for the application of the procedure to field data for both cough recognition and localisation.

The first data set consists of recorded sounds in laboratory conditions. Coughs from healthy animals were induced in an inhalation chamber by injecting an irritating substance, namely 0.8 moles per litre of citric acid dissolved in a saline solution (0.9% NaCl) (for more information on the installation environment and the data acquisition process see (Van Hirtum and Berckmans, 2003a). The nebulisation of citric acid stimulates the cough receptors directly resulting in coughing. In total, 11 experiments were conducted to 3 male and 3 female healthy Belgian Landrace piglets of 9–12 weeks of age and 20–40 kg of weight. The experiments were conducted with individual animals. In order to record pathologic coughs, the piglets were anaesthetised with azaperone (4 mg/kg intra-muscular (IM)), ketamin (10 mg/kg IM) and thiopental (10 mg/kg into a vein). They were treated (by intra-tracheal administration) with lipopolysaccharide from *Escherichia coli* diluted in sterile saline (100 μg/kg). A non-toxic strain of *Pasteurella multocida* (code 3301) was used to generate bronchopneumonia, a common respiratory infection in piglets (Kobish and Friis, 1996). Apart from the cough sounds, other sounds (such as screams, sneezes or metal sounds) were acquired and labelled accordingly by auditory processing (Van Hirtum and Berckmans, 2003a). Therefore, the generated data set includes individual sounds of 231 healthy coughs, 291 sick coughs, 18 screams, 19 sneezes, 31 grunts and 81 metal sounds (e.g. clanging doors).

The second and third data sets consist of field recordings in a fattening compartment of a pig stable in Milan, Italy. The dimensions of the stable were 14 m by 21 m with a height of 3.75 m against the walls and the roof had an inclination of 30%. The floor was totally slatted and the total area was divided into 16 boxes, eight on each side of a central path along the length of the stable. There were 350 animals in the stable, with an average of 22 pigs a box. The pigs were aged three months and were fattened from 26–35 kg at the beginning of a cycle to reach 90–100 kg in 90 days. The genetic line was a cross between landrace Italian X large white X duroc and serve for the Parma ham production. The acquisition was conducted in May 2005.

The second data set consists of triangle sounds recorded by 7 microphones in an empty stable and were produced at a height of 1 m above the ground at predefined positions. These were used to evaluate the accuracy of the localisation algorithm. The third data set consists of continuous recordings by 7 microphones (of 2 hours and 20 minutes duration each) in the pig house during normal operation and was used to test both the recognition and localisation algorithms on field

data. The sick animals in this case were suffering from pleuropneumonia from *Actinobacillus Pleuropneumoniae*.

For the sound registration 7 electret microphones (Monacor ECM 3005) were used that have a frequency response of 50–16000 Hz and were connected via preamplifiers (Monacor SPR-6) to an eight channel analogue to TDIF interface unit (Soundscape SS8IO-3). The Soundscape unit, which allows for simultaneous recording of 8 channels, was connected via a TDIF cable to a PCI audio card (Mixtreme 192). All recordings were sampled at a sample rate of 44.1 kHz with a resolution of 16 bit. All microphones were hanging in the stable at a height of 1,20 m at the positions depicted as big dots in Figure 2.

Classification and localisation

The time domain characteristics of the sound signals are used to form a cluster where the sick cough sounds belong. To present the classification properties of the proposed technique, 5 randomly selected sick cough sounds from the first data set are used to form the *sick cough* cluster. The classification procedure is subsequently applied to the whole first data set resulting in 92% correct classification ratio (with 2.8% false positive identification ratio), while 88% of all the sick cough sounds are identified (i.e. 12% false negatives).

The localisation algorithm is based on the "Time Difference of Arrival" (i.e. the difference in time for a sound to arrive at different microphones) of the sound to the microphones that are placed in predefined positions in the pig house. The proposed technique is applied to the second data set in which the triangle sounds were produced in known positions. The algorithm detected the origin of the sounds with an average accuracy of ±61cm and ±34cm along the long and short dimensions of the pig stable respectively. This level of accuracy is considered acceptable for the present application since it is important to identify the pen in which the sick animal is located and not necessarily the sick animal itself.

Analysis of field data

The flow chart for the proposed application for cough recognition and localisation is shown in Figure 1. It comprises mainly of three sub processes, namely the sound extraction, the cough recognition and the localisation, that are presented in the following.

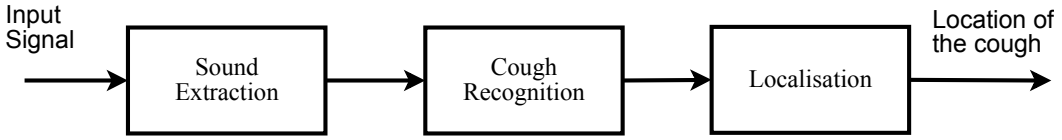

Figure 1. Flow chart for the procedure of sick cough recognition and localisation

Table 1. Results of the cough recognition algorithm on the field data

Microphone	Total Number of Sounds indicated by the algorithm to be sick coughs	Number of sounds that were correctly identified as sick cough	Percentage of correct identification
1	88	64	73%
2	101	76	75%
3	41	33	80%
4	40	37	93%
5	35	29	83%
6	47	38	81%
7	37	31	84%

To reduce the computational load for the online application, and since the pig vocalizations do not include frequencies higher than 10 kHz (Van Hirtum and Berckmans, 2001) the data are downsampled to 22.05 kHz. Furthermore, to deal with low frequency noise, and since the sick cough signals that need to be recognized do not have considerable low frequency components (Van Hirtum and Berckmans, 2001, 2003), the signal is initially bandpass filtered. For this, a 10th order Butterworth filter with pass band 2–10 kHz is used.

RESULTS AND DISCUSSION

The cough recognition and positioning procedures described above are applied to the continuous recordings of the third data set. The first five cough sounds from each microphone were manually extracted from the continuous recording, labelled by auditory means and used as a training set to define the cough clusters for every microphone. It is necessary to construct a different cluster for every microphone since the acoustics of the compartment have a different effect on each microphone mainly due to the location of the microphones (four are located in the corners, two against just one wall and one in the middle of the compartment). The result is presented in Table 1.

On the correctly recognized cough sounds, the positioning algorithm is applied and the result is presented in Figure 2. It can be seen that a clear *cough hazard* can be located at the bottom left corner and another one might be occurring at the middle left side of the pig house. Although coughs are detected in other places of the pig house, it is clear that not all of them can be considered as a hazard and an alarm should occur only for the two mentioned areas. Since a sick cough would be repeated, coughs detected in areas others than the ones mentioned, could be the result of algorithm failure or isolated cough events that pose no threat to animal welfare in the pig farm.

To the authors' knowledge, this is the first application for combined cough recognition and positioning presented in the relevant literature and can pose as a starting point for further research. The results seem promising, although there are still issues to be considered. At this point, if a cough is identified in the recording of a single microphone, the recognition is considered correct and the cough is located. A possible enhancement could be based on a trade off between sensitivity and correct recognition perhaps requiring a sound to be identified as a cough from more than one microphone.

The classification based on the time domain characteristics of the sounds is a simple technique and the computational load is held to a minimum. It is clear that a connection between them and the cough production procedure needs to be made to theoretically justify the results presented here. It is clear that superior techniques for pig cough recognition from continuous recordings exist (Van Hirtum and Berckmans, 2003a). However the repetition of sick coughs in a pig house allow for the application of the proposed technique. It is computationally effective and provides the necessary accuracy of the specific application. Another issue that still remains to be studied is the effect of environmental noise to the time domain characteristics of the sound and how this affects the accuracy of the proposed technique. It is known however that training of the algorithm needs to take place before applying it to a new environment, but the accepted level of the surrounding noise still needs to be determined.

Since the application of pig cough localisation is in pig houses, the accuracy of the algorithm to be used need not be very high, considering animal movement and that the objective is to identify the area that needs to be treated. Hence a simple sound initiation detection technique for determining the TDOA is applicable.

CONCLUSION

An automatic algorithm for sick cough recognition and localisation has been presented. Both the recognition and the localisation processes are based on simple and computationally effective algorithms, making it an attractive solution for fast observation of the spread of a disease in a pig house.

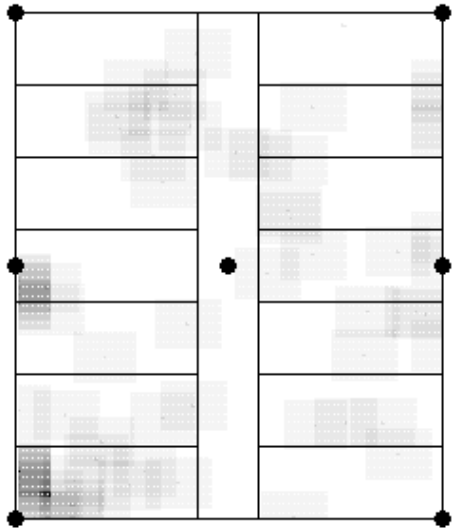

Figure 2. Representation of the pig housing environment where the dark areas indicate the positions of sick animals as a result of the proposed algorithm. Two possible health hazards emerge (at the bottom left corner and at the middle of the left wall)

The sick cough identification algorithm is based on analysis of the time domain characteristics of the sound signal and has been shown to be a potential classifier for sick pig cough sounds. Although the nature of the connection between the time domain characteristics and the physical system parameters for pig vocalizations is not yet known, the present results indicate that such a connection does exist and will be the subject of further research.

It is suggested that the present application can be used to continuously monitor animal health and that it can help in the improvement of animal welfare in pig houses. This can lead to early identification of sickness in a pig compartment and selective treatment of sick animals in the pens of the identified hazard.

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POSTER PRESENTATIONS

A WHOLE FARM ANALYSIS OF GOAT PRODUCTION SYSTEMS IN NORTH SINAI, EGYPT

Alsheikh, S.M. and El-Shaer, H.

Desert Research Center, P. O. Box 11753, Mataria, Cairo, Egypt

SUMMARY

The present study was carried out in the North Sinai region, 320 km North East of Cairo. Data were collected as a part of the project sponsored by MERC, USAID. Three goat production systems were identified in North Sinai according to water source and type of feeding or grazing. The goat production systems classified were System-1; extensive rain fed (S1). System-2; semi intensive production where water source is the rain and animals received supplementary feeding besides grazing (S2). System-3; intensive irrigated production system (S3). The study was carried out on 234 farms during the agriculture year 2001–2002. The data were statistically analyzed to test the effect of the three mentioned systems on two economic indicators, Inter rate of return (IRR %) and return per animals (RPA) per LE. The statistical analysis showed significant ($p<0.01$) effects on these economic indicators in the three studied systems. The highest IRR % and RPA per LE was scored for system 3, while the lowest was scored for system 1. Linear programming LP model was used to optimize gross margin of each system. The optimal LP model suggested for S1 system an increase in maximum grazing area from 18 feddans to 28 feddans in winter and from 24 feddans to 32 feddans in summer and herd size from 20 head to 30 head. For S2 system, the suggested increase was 8 feddans to 17 feddans in winter and from 12 feddans to 20 feddans in summer for grazing area and from 29 heads to 40 heads for herd size. On the other hand, the largest income for the LP model was noted when the herd size was maintained at 70 heads in S3. It could be concluded from the LP model that both grazing area and available cash resources were the limiting resources while labour was not. In goat production systems in North Sinai, goat activities contribute substantially, about 14–25%, to the total farm gross margin. Statistical and LP analyses showed different results, with highest return in system 3 in statistical analysis to highest return in system 2 in LP analysis.

Keywords: production systems, linear programming, goats, grazing

INTRODUCTION

In Egypt, raising livestock is an important component of the agricultural sector. Among livestock types, small ruminants contribute a greater share in numbers and output than they have elsewhere in the world. In addition, the total number of goats in Egypt is about 5 millions heads and there were 59 heads of goats per 100 feddans (MoALR, 2004, El-Shaer, 1999). Goats constitute an important animal resource under arid and semi-arid conditions. Owners, looking for the best

possible way for handling and allocating their resources, usually use their experience for maximizing their farm income. However, sometimes, their experience does not guarantee optimal results. Accordingly, linear programming (LP) could be used as an effective technique to address the limited production resources among different agricultural (cultivation and livestock) activities to provide optimal results for these owners (Alsheikh, et. al., 2002).

This study adopted linear programming (LP) technique to determine the optimum situation of the different three goat production systems in North Sinai of Egypt. In addition, comparison was made between the suggested structure obtained from the LP model and the actual structure in the three studied systems.

MATERIALS AND METHODS

Data and technical coefficients: The present study was carried out at the North Sinai region, 320km North East of Cairo. The target area extends about 150km in length with approximately 50km depth. The annual rainfall ranges from 100–200 mm (winter season) during October to March (Galal et. al., 2002). The questionnaire data were collected during 2001–2002 as a part of the USAID/Middle East Regional Cooperation (MERC) program project titled "Multinational approaches to enhance goat production in the Middle East". A total of 234 owners were involved in a specific questionnaire sheet covering all possible agricultural, social and economic information. Three goat production systems ware classified according to water source and the type of feeding and grazing. System-1; extensive rain fed (S1). System-2; semi intensive production where water source is the rain and animals received supplementary feeding besides grazing (S2). System-3; intensive irrigated production system (S3). Technical coefficients of the three goat production systems are presented in Table 1.

Economic indicators: Two economic indicators were considers. The first was the internal rate of return (IRR), defined as the rate of return that would be achieved on all farm resource costs, where all benefits and costs are measured in economic prices and calculated as the rate of discount for which the present value of the net benefit stream becomes zero, or at which the present value of the benefit stream is equal to the present value of the cost stream at interest rate of 10%. The second economic indicator considered was return per animal (RPA) defined as the gross margin divided by number of animals.

Statistical analysis: Data were analyzed using SAS system for Windows (1998). The models used to study different factors potentially affecting IRR and RPA. The mathematical details of the model are shown below.

$$Y_{ij} = \mu + S_i + F_{j(i)}$$

where,

Y_{ij} = the observation on the j^{th} farm, within the i^{th} system;
μ = overall mean;
S_i = the effect of system, i =1,…3; and
$F_{j(i)}$ = the effect of farm within system, j =1,…234. The farm was considered as the model error, assumed to be normally and independently distributed with mean 0 and variance σ^2_F.

Mathematical LP: LP model was done using GAMS (2000) software to compare the efficiency of the three studied production systems under the following assumptions:

1. The optimization LP function was used to maximize the farm gross margin, which was calculated by subtracting the variable cost from gross output.
2. LP model constraints included available cash resources (ACR), which was assumed to be equal to the gross output, labour, grazing area, doe productivity and feeding requirements (Table 1).
3. Variable costs in both S2 and S3 included feeding requirements, veterinary services, labour and other miscellaneous costs. While, in S1 included only labour cost for one shepherd.
4. Gross output (GO) was calculated as: GO = kg live body weight of does sold in S1 and sold fattened kids in S2 and S3 each multiplied by 15 LE (farm gate price in 2002).
5. There is no dynamic relationship between grazing and growth performance.

Table 1. Technical coefficients of the three goat production systems in North Sinai

Items	System		
	S1	S2	S3
Biological coefficients			
Av. Herd size (head)	20	29	45
Av. Litter size (kids/doe/kidding)	1	1.2	1.6
No. of weaning kids/doe/year	1	1.14	1.24
Yearling rate (no. of kids alive at yearling/doe)	0.9	1.22	1.90
Average weaning weight of kid (kg)	10	9	8
Average kg weaned / doe / year	10	13	18
Average kg sale / kid	15	21	30
Average kg sale / doe / year	13.5	25.6	57
Replacement rate (yearling does)	0.15	0.2	0.25
Saleable kid /doe / year	0.75	1.02	1.65
Net doe production at sale (kg/ doe / year)	11	21	50
Kg live body weight of fattened kids/ doe/ year	NP	15	35
No. of kg of concentrate/ kg live body gain	4	5	6
No. of kg of concentrate for fattened kids / doe / year	NP	50	150
Economic coefficients per farm (LE)			
Gross output (GO)	3200	9000	21000
Variable costs (VC)	2400*	5600	12500
Gross margin (GM)	1200	3400	9500

S1: Extensive rain-fed production system.
S2: Semi intensive production system.
S3: Intensive irrigated production system.
NP: Not practical.
* Variable cost in S1 was assumed that one shepherd obtained 200 LE as monthly salary.

RESULTS AND DISCUSSION

Statistical model solution: The intensification form system to anther depends on higher kidding rates, lower kid mortality and higher sale weights of fattened kid. The statistical analysis showed significant effects on the two studied economic indicators in the three studied systems (Table 2). Higher level of significance ($p<0.01$) was detected for IRR and RPA indicating that systems

responded differently to the owner activities. The highest IRR percentages and RPA per LE was scored for system 3, while the lowest was scored for system 1.

Table 2. Least squares means (LSM) and standard errors (±SE) for the impact of three goat production system on inter rate of return (IRR %) and return per animal (RPA) per LE

	IRR (%)				RPA, LE		
Source of variation	No.	LSM	±SE		No.	LSM	±SE
Systems		0.07**				0.05**	
S1	52	0.13a	0.10		52	0.01a	0.20
S2	87	0.15a	0.11		87	0.02b	0.01
S3	95	0.19b	0.06		95	0.03c	0.01
Farm (System)	0.007 (231) df				0.007 (231) df		

Farm (System) used as an error term.

Mathematical Linear Programming (LP) solution: The actual situation and optimal LP output solutions are shown in Table 3 and Figure 1. The optimal LP solution suggested that, owners should increase the average herd size from 20, 29, 45 head to 30, 40 and 70 head in S1, S2 and S3, respectively. Also, grazing area should be increased from 18 feddans to 28 feddans in winter and from 24 feddans to 32 feddans in summer in S1. In S2, the grazing area should be increased from 8 feddans to 17 feddans in winter and from 12 feddans to 20 feddans in summer. In addition, labour should be increase by 100% in the three studied systems. Moreover, the raw gross margin in S3 was higher than the other two studied systems (S1 and S2) while, the relative gross margin to actual situation was improved by about 43%, 47% and 45% in S1, S2 and S3, respectively. Also, the relative return per head was improved by about 14%, 25% and 14% in S1, S2 and S3, respectively. These results indicated that S2 showed higher economic efficiency than other two systems (S1 and S3) in both relative gross margin and return per head. Moreover, the S2 system used the highest number of labour compared with other two systems as it included two activities i.e. grazing and fattening kids. On the other hand, the S2 system would help to reduce the feeding requirements through animal grazing. So, it could be recommended to owners in other different goat production areas in North Sinai region to follow this system in order to improve their income by 11% per year.

Table 3. Actual situation (A) and linear programming (LP) solutions for the three studied goat production systems in North Sinai of Egypt

Item	System I			System II			System III	
	A	LP		A	LP		A	LP
Biological output								
Av. herd size (head)	20	30		29	40		45	70
Gazing area (feddan)								
Winter	18	28		8	17		–	–
Summer	24	32		12	20		–	–
Labor (person-day)								
Winter	1	2		2	4		1	2
Summer	1	2		2	4		1	2
Economic output								
ACR (LE)	3200	5000		9000	9000		21000	21000
Gross margin (LE)	1200	2100		5600	10320		9500	17230
Return per head(LE)	60	70		193	258		211	246

Values were round to the nearest integer.

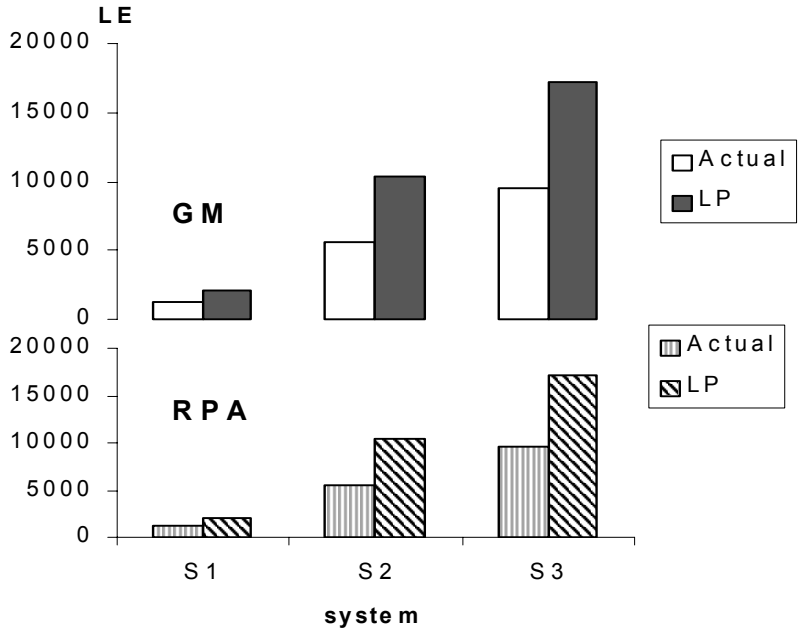

Figure 1. Gross margin (GM) and return per animal (RPA) per LE for actual and LP solution for the three studied systems.

CONCLUSION

The three studied systems had positive significant ($p < 0.01$) effect on the two studies economic indicators. The degree of impact differed among the three studies systems where system 3 showed the highest impact on IRR % and RPA per LE. LP model showed that both grazing area and available cash resources were the limiting resources while labour was not. In goat production systems in North Sinai, animal activities contribute substantially, about 14–25%, to the total farm gross margin.

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THE ASSESSMENT OF ANIMAL MAINTENANCE IN ECOLOGICAL FARMHOLDS IN POLAND IN THE VIEW OF DOMESTIC REQUIREMENTS[1]

Bartkowiak, A.[1], Bartkowiak, P.[2], Dobrzański, Z.[1] and Roman, A.[1]

[1] Wroclaw University of Environmental and Life Sciences, Department of Animal Hygiene and Ichthyology, Chelmonskiego 38 C, 51-630 Wroclaw, Poland, ania@ozi.ar.wroc.pl;
[2] Poznan University of Economics, Faculty of Management, Department of Production Economics, Al. Niepodległosci 10, 60–967 Poznan, Poland

SUMMARY

The aim of the work is the assessment of animal maintenance conditions, feeding and prophylaxis in certified ecological farmholds on the basis of questionnaire testing. Basing on the results obtained it can be stated that considerable part of the farmholds meet most of criteria. Livestock population amounted average 1,36 large heads per the parity hectare, but per 1m^2 of the area accessible for animals was mostly much smaller. Most of those buildings possess windows and gravity ventilation. Each animal was kept on bedding, yet not all of them had an access to water and most animals were tied. Every farmhold fed animals basing on this own fodder. Veterinary assistance depended on the basic treatments.

Keywords: ecological farmholds, assessment of animal maintenance, domestic requirements

INTRODUCTION

In the recent years there has been observed higher social awareness regarding the needs of health protection through the consumption of high quality and healthy food. This can be proved by ecological agriculture as sustainable system of food production. The latter one has been undergoing dynamic development in Poland, which can be proved by considerably increased number ecological farmholds, as well as the areas of arable land for example, until 2005 there was recorded 6 times higher number of the mentioned farmholds in comparison to 2000. In 2000 there were 388 of them, while in 2005 the number of ecological farmholds ranged 2050 [1].

The conversion of traditional farmholds into ecological ones means, however, meeting certain requirements. This refers both to plant and animal production [2]. Ecological farmholds breeding animals have to meet appropriate criteria to ensure animal welfare, as well as the protection of natural environment. According to legal acts being in force in our country, significant criteria are, first of all, animal origin, maintenance conditions, including animal housing, feeding and veterinary assistance [3,4,6].

The aim of the work is the assessment of animal maintenance conditions, feeding and prophylaxis in certified ecological farmholds on the basis of questionnaire testing.

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MATERIAL AND METHODS

The material for investigation was the results obtained on the basis of directed interviews which involved the farmers running certified ecological farmholds. The total number of questionnaires sent was 120. On average, 2 questionnaires were sent back from each voivodship, which resulted in statistical questionnaire return ranging 27,5%. Eventually, complex questionnaire investigation involved 33 certified ecological farmholds and referred to such data as agriculturally developed land, including the arable one, livestock population, animal maintenance conditions, prophylaxis and the ways of veterinary treatment.

RESULTS

The investigations proved that livestock population in those farmholds amounts average 1.36 SD per parity hectare. Taking into account all the farmholds, 46.2% of them kept the number of animals ranging from 0.5–1.5 SD per parity hectare, while 34% of the respondents possessed smaller number of animals, i.e. 0.006–0.46 SD per parity hectare and 19.2% more, i.e. 1.67–2.95 SD per parity hectare.

Nearly each farmhold dealt with breeding and rearing of dairy cows and laying hens. In 12 farmholds there were fatteners, and, occasionally, other species of animals. The number of cows ranged from 1 to 4 heads, in 5 farmholds there were kept 5–16 heads and in 1 farmhold there were found 40 animals. In the case of fatteners the number of animals did not exceed 14 heads and only in 1 farmhold there were 80 fatteners. The highest number of farmholds possessed from 10 to 50 laying hens and only in 3 farmholds there were 100, 300 and 340 hens. In the case of the latter farmhold this is a local breed – green-legged partridge. Broilers were recorded in 2 farmholds, about 30 heads in each, and in 6 farmholds there were rabbits numbering from 3 to 60 heads. Average number of animals, calculated over 1 farmhold, proved that the highest number belongs to sheep – 72 heads, about 53 heads – for laying hens, while the lower number features horses – about 7, and cows – about 5 heads (Fig. 1).

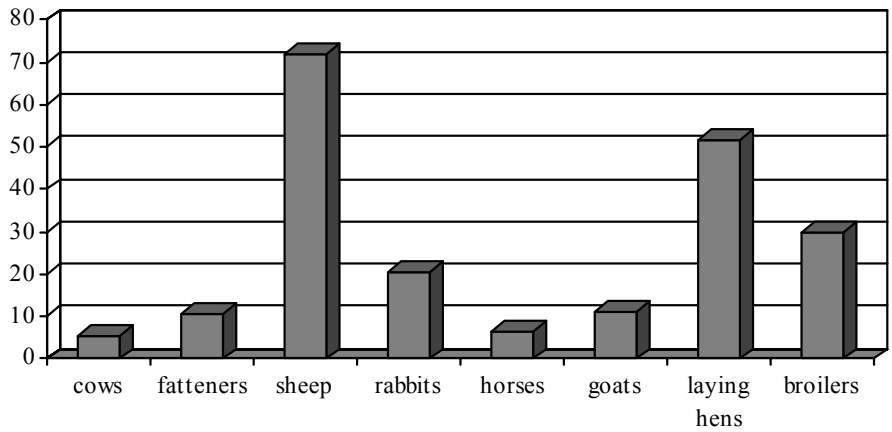

Figure 1. Average number of animal in ecological farmholds

Ecological farmholds also possess other animals, though in much smaller number. Apart from the animals mentioned above, there were recorded calves, heifers, bulls, geese and bees, with cattle providing for the largest part (Tab. 1).

Table 1. The number of farmholds possessing other animals (physical heads)

Animals	Number of households	Range	Average
Cattle:			
Calves	8	1–7	4,1
Heifers	7	1–6	2,7
Bulls	6	1–5	2,0
Sows	1	1	1
Geese	3	3–6000	2024,7
Bees	1 apiary	60 (beehive)	60 (beehive)

Part of the respondents, i.e. 37.9% confirmed that they planned to increase livestock population, 6.9% farmers were going to decrease it, while 55.2% wanted to maintain the already existing of animals in their farmholds. The area of barn building amounts 70–2400 m^2 and the area of buildings for animals 40–2400 m^2. Stocking density in 14% ecological farmholds corresponds to appropriate number of animals per 1m^2 of building area, while in the remaining ones stocking density is too low (79%) or too high (7%).

The barn in the highest percent of ecological farmholds were built 20–36 years ago (57.1%). New buildings (3–14 years old) constitute 10.7%, those 40–65 years old – 17.9% and the ones built before Second World War (70–100 years old) provide for 14.3%. Those barns were built from bricks – 72,7%, wood – 6.1%, while the remaining ones (21.2%) from other materials such as: hollow bricks, suporex, stone, gas concrete, calcium-silicate bricks. In 1 farmhold, apart from a brick building, there was a wooden roof shelter for animals.

Nearly all buildings possess windows (96.8%). Gravity ventilation is in 75% buildings and the remaining ones featured mechanical ventilation (9.4%) and the mixed type (15.6%) – Fig. 2. In each barn animals were kept on bedding and regular access to water have 73.3% of animals, while in 55.6% of farmholds animals are tied, though some farmholds introduced both tied and loose animal keeping system. Animals can use an outside run in 86.7% of farmholds and 96.7% use pastures. Besides, in farmholds breeding dairy cows, hand milking takes place in 66.7% of them and the remaining ones apply mechanical milking system.

Examination results regarding animal feeding showed that nearly all farmholds use feeding stuff produced solely by them. That kind of food mainly consist of: corn mixtures, corn waste, leguminous plants, crushed meal, grass hay, grass silage, root crops (potatoes, fodder beet, carrot, parsley) and other, e.g. fodder pumpkin. In 4 farmholds they use 10–15% purchased feeding stuff like: concentrates, premixes, dehydrated forage, rape crushed meal, crops, linseed. Among feeding stuff supplements salt-lick is most commonly used – nearly 79% of farmholds, as well as fodder chalk (31.6%) and other: eco-minerals, eco-concentrate, eco-premix and vitamins. Herb extract and charcoal also belong to eagerly used supplements.

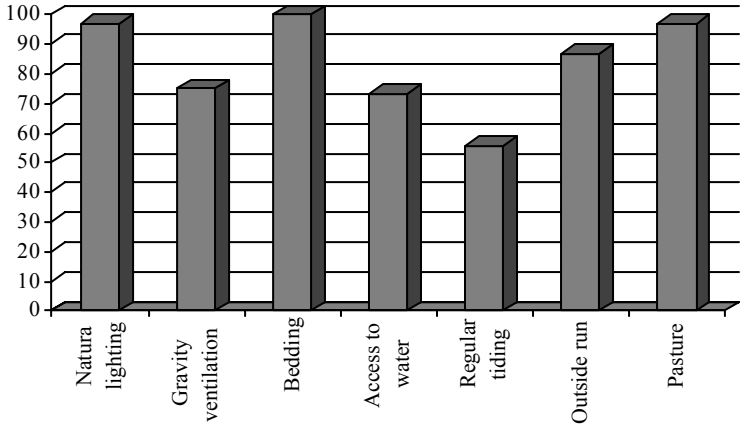

Figure 2. The conditions of animal maintenance in ecological farmholds (%)

On the basis of the investigation it is possible to state that veterinary assistance consisted in the treatments like hoof paring, sheep disinfestations and obligatory vaccination. Most respondents claimed there was no need to introduce any pharmacological means as animal state of health and productivity mainly depend on feeding and providing the possibility of animal being on the go on fresh air. Some farmers use balm in cows to cure udder inflammation. As far as antibiotics were considered, only one respondent stated that he used antibiotics if needed. Milk production by cows in ecological farmholds showed relatively different level, from 2500 to 9750 litres per year (Tab. 2). Hen laying ranged from 50% to 95%.

DISCUSSION

Animal production constitutes an integral part of any ecological farmhold. The number of animals has to be closely related to their accessible area. This correlation aims at avoiding overgraze and pasture erosion, as well as using manure to prevent negative consequences on the part of the environment, including soil contamination and water pollution, both surface and underground ones [3,4]. Stocking density should, therefore, amount 0.5–1.5 SD/partition hectare. Moreover, appropriate selection of animal species and breeds, animal origin, maintenance conditions including minimum area, proper treatment of animals, as well as feeding, prophylaxis and medical treatment are also of considerable importance [3,5,7].

Appropriate selective breeding is intended to ensure satisfactory level of animal production, maintaining, at the same time, diversity of plant production. Animals should, first of all, feature good state of health and be suitable for making use of farm-produced fodder. They also should originate from farmhold own breeding or come from other ecological farmholds. Animals obtained due to genetic engineering or fetus experiments are not accepted.

It is very important for ecological farmholds to provide appropriate feeding and maintenance of animals. Aiming at animal welfare, outside runs and pastures should be accessible and in indoor breeding. Tiding systems or trainers must be obviously excluded. To ensure suitable microclimate in barns lighting (preferable natural lighting) and gravity ventilation should be

introduced. Animals should have regular access to drinking water and fodder. Feeding should be based on ecologically produced fodders. Special requirements are set by prophylaxis and veterinary treatment as conventional methods can be applied only when animal life is endangered, to prevent suffering on in case drugs are not accessible [3,4,5,7].

Analysis of the results proved that most farmholds subjected to investigation meet basic requirements regarding stocking maintenance, feeding and prophylaxis. In more than 46% the number of animals fulfilled advisable norms (0.5–1.5 SD/partition hectare). Yet there are still farmholds of too low or, to lesser degree, too high stocking density, which can lead to disturbances of fodder-fertilizer balance in ecological farmholds. It should be noticed that in the examined farmholds there dominate milk and egg production, to a lesser degree pork production, while other animal production, e.g. goat milk, poultry and rabbit meat provide for the lowest percentage. Only a few farmholds run broiler and fowl production. As it results from our investigation, the area accessible for animals has not been completely used, which can also effect on inside microclimate. This situation results from, among the others, lack of subsidies for animal production.

Animal maintenance conditions are relatively different in particular farmholds, although most requirements are met in this respect. Yet not all the farmholds had adjusted to the respective criteria, namely about 27.7% of farmholds do not provide their animals with regular access to water, 55.6% of farmholds use animal tiding system, as well as not all animals can enjoy outer runs or pastures. The fact that nearly all farmholds use fodder originating from their own production, which, in turn, is according to the respondents a source of animal good state of health and high productivity of animals kept, seems to be quite satisfactory. The mentioned farmers, for the same reason, do not need to use drugs and veterinary assistance consist mainly in basic treatment, i.e. hoof paring, disinfestations and obligatory vaccination.

CONCLUSION

Concluding, it should be stated that increased number of ecological farmholds in Poland can be regarded as quite considerable one. Yet not all farmholds have been converted to meet domestic requirements. Moreover, numerous farmers do not consider it necessary to cooperate with academic or agricultural advisory centres, which would eagerly provide that kind of services. The mentioned cooperation would allow to easier and more rapidly adapt ecological farmholds to home requirements, which would additionally increase the interest in ecological products among the Polish and foreign consumers.

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CHARACTERISATION OF COUGH SOUNDS TO MONITOR RESPIRATORY INFECTIONS IN PIGS

Ferrari, S.[1], Silva, M.[2], Guarino, M.[1] and Berckmans, D.[2]

[1] Department of Veterinarian Sciences and Technologies for food Safety, Faculty of Veterinary Medicine, University of Milano, Via Celoria 10, 20133 Milano, Italy, sara.ferrari@unimi.it; marcella.guarino@unimi.it; [2] Department of Biosystems, Division M3-BIORES: Measure, Model & Manage Bioresponses, Catholic University of Leuven, Kasteelpark Arenberg 30, 3001 Heverlee, Belgium, mitchell.silva@biw.kuleuven.be; daniel.berckmans@biw.kuleuven.be

ABSTRACT

Cough is the element for monitoring and diagnosis of respiratory disease cause of mortality and loss of productivity in pig houses. In order to prevent as much as possible the outbreak of such diseases the aim of this research is to describe acoustic features of cough sounds originating from infections due to Actinobacillosis and Pasteurellosis and to compare them with healthy cough sounds provoked by inhalation of citric acid. The acoustic parameters investigated are peak frequency [Hz], duration of cough signals. Sound analysis provides physic acoustic features that can be used as tool to detect and label cough in an automatic monitoring system applied in farms.

Keywords: cough sound, respiratory disease, Pasteurellosis, Actinobacillosis, bio-acoustics

INTRODUCTION

Respiratory pathologies, together with enteric diseases, are frequent in pig husbandry and cough is their principal symptom. The importance of these diseases must be seen on economical and sanitary level for their high veterinarian intervention costs and to a loss of profit due to higher mortality and drop of production due to reduced feed conversion and growth rate. It is also ascertained that detecting illness in individual animals and providing individual care, or group-by-group mass therapy in response to illness, are both not very effective and are costly. Due to their numerousness and incidence in farms it is crucial to investigate cough sounds with the aim of understanding respiratory diseases and use bioacoustics for real time monitoring purposes.

The importance of coughing as a means of prognosis does not refer only to humans, but also to animals. It has been shown that pig vocalisation is directly related to pain and classification of such sounds has been attempted (Marx *et al.*, 2003). It is also common practice by veterinarians to assess cough sounds in pig houses for diagnostic purposes. In this regard, there have been attempts to identify the characteristics of coughing in animals (Moreaux *et al.*, 1999, Van Hirtum, 2002a) and automatically identify cough sounds from field recordings (Aerts *et al.* 2005; Van Hirtum & Berckmans, 2003a, 2003b).

The aim of this work, by comparing different sick cough and a healthy one, is improving the labelling of coughs giving physic features to specifics sounds, those characteristics will be used as inputs in an automatic alarm system based on an algorithm that will recognize cough sounds from

an installation in a farm and will provide early warning to the farmer on the welfare status of his herd. Automatic real time monitoring and early detection of these respiratory pathologies can be applied in intensive farms considering the high number of animals hosted. This can reduce the spread of the disease, save costs and provide information of how to face, in terms of bio security, the problem of prevention and spread of respiratory pathologies.

MATERIAL AND METHODS

In this work we present a comparison between cough sounds of healthy and sick animals made on a database of healthy coughs induced in laboratory conditions and sick coughs collected in field conditions in two affected pig farms fattening compartments. Both the pig farm breeding serve for the Parma ham production. The Pasteurella sick pigs, a hybrid strain Landrace x LW + Danish Duroc boar, were at the beginning of the fattening period weighing around 40 kg. The diagnosis of a veterinarian and the serologic results assured that those animals were sick, affected by pneumonia due to *Pasteurella Multocida*. The pigs suffering from infection due to *Actinobacillus Pleuropneumoniae*, were aged three months and were fattened ranged from 26–35 kg at the beginning of a cycle to reach 90–100 kg in 90 days. The genetic line was a cross between landrace Italian X Large White X Duroc. The healthy cough was induced by inhalation of citric acid in Belgian Landrace x Duroc piglets weighing between 20 and 40 kg (Van Hirtum, 2003).

For the sound acquisition 7 microphones (Monacor ECM 3005) were used with a frequency response of 50–16000 Hz, connected via preamplifiers (Monacor SPR-6) to an eight channel analogue to TDIF interface unit (Soundscape SS8IO-3). The Soundscape unit, which allows for simultaneous recording of 8 channels, was connected via a TDIF cable to a PCI audio card (Mixtreme 192). All recordings were sampled at a sample rate of 44.1 kHz with a resolution of 16 bit. All microphones were hanged in the stable. The healthy cough sounds were caused by a temporary irritation of the upper respiratory tract. On the contrary the sick ones were caused, in Pasteurellosis case, by a deep bacterial infection of the lungs since the infectious process starts at the alveolar bronchiole junction producing exudates and in the Actinobacillosis disease by a lung lesion with large red-blue areas in the upper diaphragmatic lobes with an overlying pleurisy. For recording and labelling of the cough sounds in both lab and field Adobe Audition 1.5 was used, for the signal processing Matlab 7.1 and SAS statistical package 2004 for the statistical analysis.

Analysis of the collected data

The characteristics of the cough sounds were identified in both time and frequency domain. The spectrograms of the coughs where built using a Hanning windowing function with a length of 40 ms and 20 ms overlap. The signal from the microphone was band pass filtered between 100 Hz and 10800 Hz to get rid of the low frequency noise. A comparison between healthy and sick coughs sounds have been made by considering the duration of the signal and the energy in the frequency content. The duration of a single cough, the number of hits and the time between the coughs in a cough attack were considered. This is illustrated in figure 1.

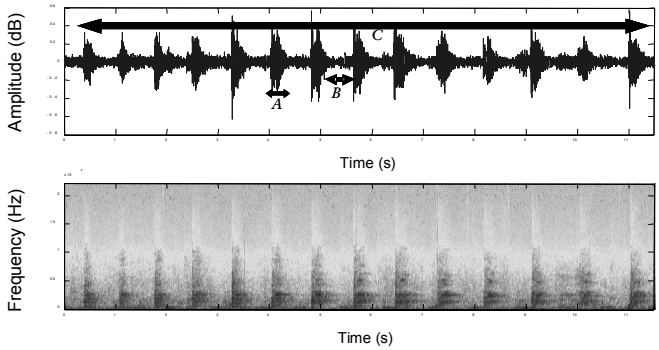

Figure 1. Pig cough attack (14 hits showed) represented in time domain (above) and in frequency domain (below).The arrows indicate the parameters studied. a) length of a cough, b) time between two cough, c) total length of the cough attack.

These parameters have been counted with auditive and visual observation on the sound spectrum by the operator using Adobe Audition program. For every cough signal the peak frequency (frequency with maximal energy content) was calculated. The analysis of variance (SAS; GLM) has been done on both the length of single coughs and cough attacks among the three classes of coughs to evaluate the certain interclasses distinction in time and frequency domain.

RESULTS

During the recording sessions we collected 851 coughs from pigs affected by Pasteurellosis and 186 coughs coming from pigs sick of Actinobacillosis coming from respectively 91 and 26 cough attacks.

The comparison made with the database of healthy coughs inducted by inhalation of Citric Acid investigated first of all the duration of the sounds

The average number of coughs in a cough attack was 13 for healthy coughs and 9 and 7 for Pasteurella and Actinobacillus ones (table 1).

The results, in terms of number of single coughs and attacks, duration, mean duration and standard deviation of the signals, are illustrated in tables 1 and table 2.

Table 1. Number of cough attacks and single coughs in the collected database

Type of cough	Nr. attacks	Nr.coughs	Min nr.coughs in attack	Max.nr. cough in attack	Mean number of coughs
Healthy	11	149	4	22	13.54
Pasteurella	91	851	5	25	9.35
Actinobacillus	26	186	3	19	7.15

Table 2. Duration of both cough attack and single sound signals, standard deviation of mean duration of single coughs

Type of cough	Mean duration attack (s)	Mean duration single cough (s)	DS single coughs
Actinobacillus	5.17	0.53	0.70
Healthy	8.61	0.43	0.13
Pasteurella	6.77	0.67	0.2

Concerning the differences in length of the three classes of single coughs and attacks investigated Variance analysis (ANOVA) was performed on the collected data using SAS statistical package (GLM procedure, 2004). The results show highly significantly differences among the classes ($P<0.001$) and lead us to consider the length of these signals as a tool to distinguish sounds. The ANOVA results among the duration of the three classes of cough attack show that the length of the coughs attack has a significantly difference between Healthy and Actinobacillus ($P<0.0387$) and between Actinobacillus and Pasteurella ($P<0.0493$) but not between Healthy and Pasteurella ($P<0.3418$).

The analysis lead over peak frequency of the single cough shows that lung diseases lower the peak frequency of the cough. There is a significant difference between peak frequency of coughs originating from Actinobacillus and Healthy cough sounds. The range for healthy coughs is between 750 Hz and 1800 Hz for peak frequency. For the two lung disorders this is between 200 Hz and 1100 Hz (table 3). The peak frequencies of Pasteurella coughs are clearly lower than healthy cough sounds (Healthy VS Pasteurella: $P>0.0062$; significant), but less significant than with Actinobacillus Pleuropneumoniae ($P > 0.0694$) (table 7; figure 2). Highly significant is also the diversity between Healthy and Actinobacillus coughs having $P> 0.00002$.

Table 3. Peak frequency mean among the three classes of single coughs

Type of cough	Peak frequency
Actinobacillus	200–1100 Hz
Healthy	750–1800 Hz
Pasteurella	200–1100 Hz

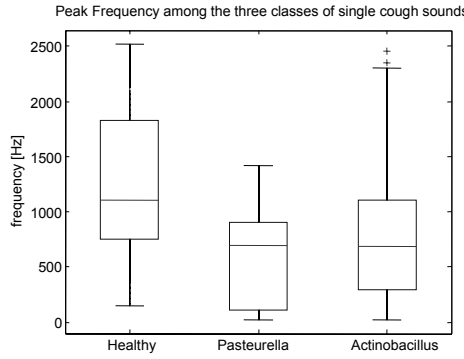

Figure 2. Boxplot of the peak frequency of the three classes of analysed coughs. The difference between the two sick coughs and the healthy one stands in a lower mean of the maximum frequency in sick coughs.

DISCUSSION AND CONCLUSION

The possibility to make a distinction between pathological and healthy cough sound by physical sound features is shown. As this work improves characterisation of the features of cough, caused by specific agents, in terms of acoustical parameters, it will be useful to improve cough sound labelling as it provides significant differences between cough arising from sick or healthy animals (Ferrari 2006, unpublished data). Literature in the past already focused on this distinction, but specifically in humans. Van Hirtum and Berckmans shown already several ways to work with pig cough, from the assessment of the cough towards vocalization (2002a), through the automated recognition of spontaneous versus voluntary cough (2002b) to the recognition of cough sound by using an algorithm for recognition in lab condition (2003a) anyway literature on acoustic features of different respiratory diseases is still unknown. In this paper sound analysis considers features like frequency energy content and duration of cough.

In terms of peak frequency of cough signal sick coughs show a significantly lower peak frequency than healthy coughs (200–1100Hz for sick and 750–1800 Hz for healthy). This is in contradiction with the findings of Korpas et al who state that frequencies of 300 Hz to 500 Hz are the most expressive in healthy human coughs whereas in cough sounds of bronchitis the bands between 500 and 1200 are the most expressive (Korpas *et al*.,1996). Sound differences in cough between humans and pigs can be explained by differences in the amount of air pushed in through the air pipe or by the dimension and characteristics of the air pipe itself. On the other hand, Van Hirtum and Berckmans (2003b) and Ferrari (2006, unpublished) showed that the fundamental frequency for healthy pig cough sounds in laboratory conditions is higher than those of sick coughs; and that's what we confirmed with our study in field conditions.

When considering the duration of a single cough, it can be seen that there is a significant difference between the two groups of cough sounds, having a mean duration of 0.53–0.67 seconds for Actinobacillus and Pasteurella, sick, coughs while 0.43 seconds was observed for healthy coughs. The trend was also observed by other authors, concluding that the duration of sick cough is longer compared to healthy one due to airways obstruction by infection and inflammation (Korpas,1996; Van Hirtum, 2002b, Ferrari 2006, unpublished data), both in humans and pigs. Concerning the duration of a single cough or a cough attack in the whole nothing is found in literature. Further analysis should be done to clarify these findings. Although a connection between the time and frequency domain characteristics and physical system parameters for pig vocalizations is not yet known, the present results indicate that such a connection exists and remains to be determined. By understanding the effect of respiratory airway inflammation and structural changes of its cell walls on cough sounds, information can be extracted about the status of the animals. Not only in laboratory conditions but also in field situations this can lead to an interesting acoustic monitoring system. The acoustics features characterizing a sick cough can be used as inputs for on-line cough counters algorithm. In this study frequency characteristics, duration of a single cough and a cough attack were compared between healthy and sick coughs. Sound analysis in field conditions provides additional, useful, non invasive objective and quantitative information about the respiratory system and is a candidate for developing automatic on-line health monitoring tool.

It is suggested that the present application integrated in an automatic detection system can be used to continuously monitor animal health and might help in advance animal welfare in pig houses.

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A RETROSPECTIVE STUDY OF EQUINE COLIC RISK FACTORS IN TABRIZ AREA IN IRAN

Hassanpour, A., Mousavi, G., and Amoughli Tabrizi, B.

Clinical Science of Veterinary Medicine of Islamic Azad university of Tabriz-Iran

ABSTRACT

A retrospective study was conducted on 10 horse farms to identify risk factors for equine colic for 1 year in Tabriz area in Iran. The association between colic and farm or individual horse risk factors related to management, housing, pasture, use, nutrition, health and events was first examined by univariate statistical analysis. Individually significant variables were used in a stepwise multivariable forward logistic regression to select explanatory factors ($P < 0.05$). Analysis was conducted at 2 levels: farm and individual horse with farm specified as a random effects variable. No farm-level variables were significant. Significant horse-level variables included: age, odds ratio (OR) = 3.1 for horses age 2–10 years compared to < 2 years; history of previous colic, OR = 2.9 relative to no colic; changes in concentrate feeding during the year (1 per year, OR = 3.3, more than 1, OR = 1.8) relative to no changes; more than 1 change in hay feeding during the year, OR = 2.4 relative to no changes; feeding high levels of concentrate (> 2.5 kg/day dry matter, OR = 5.2 > 5 kg/day dry matter, OR = 7.1) relative to feeding no concentrate; and vaccination with anthrax vaccine during the study, OR = 1.2 relative to no vaccination. Feeding a whole grain with or without other concentrate components reduced risk, OR = 0.6, relative to feeding no whole grain. Results of the study suggest that diet and changes in diet and aging are important risks for colic in a population of horses on farms.

INTRODUCTION

Colic is a term used to describe pain in the abdomen. However, there are many causes of colic some of which may be mild whilst others can be life threatening. In the early stages of colic it is not possible to tell the severity and so all cases should be treated seriously. Often the cause of colic is not be known but can include irregularities in feeding, sudden change of diet, indigestion, gas build up, too much concentrate feet or unsoaked sugar beet, eating of a substance which expands when dampened, intestinal accident, blockage, lack of water, stress, too much food and/or water after exercise, contractions, and inflammation. The risk of colic occurring is increased with high carbohydrate diets and inadequate access to hay or grass. Stabled horses are more prone to colic than grass kept horses. Recurring colic can be due to a number of more serious causes such as tumours, ulcers, and problems with one of the abdominal organs and should be investigated by a vet (9,11,and 14).

Symptoms of colic include restlessness, kicking at the belly, pawing the ground or rolling in an effort to disperse the pain, lying down more than usual, frequently standing outstretched as if to urinate, turning the head towards the flank and curling of the upper lip. A horse with colic will have a high temperature, its pulse and respiration rate will increase and it may also sweat and be off its feed. Veterinary advice should be sought immediately. Food should be removed and

nibbling at bedding should be prevented until the veterinarian arrives. The horse should be walked as this will distract from the pain and will also help prevent rolling. If it is not possible to prevent the horse from rolling the horse should be placed in an area where it can inflict little damage to itself and cannot become cast (4,7,and 8).

A regular feeding schedule, constant access to clean water, adequate forage, consistent exercise routine and the avoidance of sudden changes in diet will all reduce the risk of colic occurring(12,13).

MATERIALS AND METHODS

A retrospective study was conducted on 10 horse farms to identify risk factors for equine colic for 5 years in Tabriz area in Iran. A. The study achieved on the 260 horses of four breeds (133 Arabian, 86 crossbred, 16 Thoroughbreds, 25 Kurd) to estimate the incidence rate of equine colic and its correlation to causes or risk factors. The horses were divided by sex and age groups (young [<2 years], middle [2–10 years], old [>10 years]), with a mean of 5.1 years and median of 4 years. A questionnaire (including location of farm, breed, sex, age of animals, prevention programs, and types of feedstuffs) was filled out for each farm. The association between colic and farm or individual horse risk factors related to management, housing, pasture, use, nutrition, health and events was first examined by univariate statistical analysis. The criteria for diagnosis of colic included observation, clinical examination, and possible laboratory examination.

RESULTS

The number and combination of horses under study are showed in table 1.

Table 1. Number and combination of horses on farms

Farm	No. of horse	Combination
1	24	A:12, CB:7, T:2, K:3
2	81	A:39, CB:24, T:7, K:11
3	37	A:18, CB:15, K:4
4	16	A:7, CB:6, T:3
5	28	A:12, CB:9, T:3, K:4
6	13	A:6, CB:7
7	11	A:7, CB:4
8	12	A:8, CB:4
9	17	A:12, CB:5
10	21	A:12, CB:5, T:1, K:3

A = Arabian horse; CB = Crossbred horse; T = Thoroughbred horse; K = Kurd horse.

The severity of abdominal pain, length of pain, response to analgesics, abdominal sounds, and signs of shock were the most important signs for diagnosis. These signs along with other signs were recorded. The number of cases based on breed, sex, and age show in table 2. The mean incidence density rate of colic during 5 years was 8.85% (23/260) (including11 Arabs, 9

Crossbreds, 2 Thoroughbreds, 1 Kurd). Horses with colic were four 1–year-olds, two 2–year-olds, five 4–year-olds, seven 6–year-olds, two 8–year-olds, and three 12–year-olds).

Table2. The number of cases based on breed, sex, and age

Breed	No. of the horses with colic	sex
Arab	11 (11/133) (8.27%)	9 male and 2 female
Crossbred	9 (9/86) (10.46%)	7 male and 2 male
Thoroughbred	2 (2/16) (12.5%)	1 male and 1 female
Kurd	1 (1/25) (4%)	1 male

The highest and the lowest incidence of colic were in Thoroughbred and Kurd horses, respectively. There was significant difference between sexes (P <0 .01). The relationship between the incidence of colic and age of horse were significant (P <0 .05). The highest and the lowest incidence of colic were in 6–year-olds and 2–year-olds horses, respectively.

Nearly 78.65% of horses with colic were treated by routine procedures, including lubricants, nonsteroidal anti-inflammatory drugs (flunixin meglumine or ketoprufen), walking, and changing to a better diet if necessary. Three horses were referred to surgery.

DISCUSSION

The incidence rate of colic in the current study was 9.12% horses in 5 years. The incidence rate of colic in 14 show horse herds is reported to be 26% (1). This value also is reported to be 6.7% and 4.2% in other studies (5). Although the number of horses and the time of study are low in comparison with some other studies, this study encompassed nearly all registered horses in the city. The association between colic and farm or individual horse risk factors related to management, housing, pasture, use, nutrition, health and events was first examined by univariate statistical analysis. Individually significant variables were used in a stepwise multivariable forward logistic regression to select explanatory factors ($P < 0.05$). Analysis was conducted at 2 levels: farm and individual horse with farm specified as a random effects variable. No farm-level variables were significant. Significant horse-level variables included: age, odds ratio (OR) = 3.1 for horses age 2–10 years compared to < 2 years; history of previous colic, OR = 2.9 relative to no colic; changes in concentrate feeding during the year (1 per year, OR = 3.3, more than 1, OR = 1.8) relative to no changes; more than 1 change in hay feeding during the year, OR = 2.4 relative to no changes; feeding high levels of concentrate (> 2.5 kg/day dry matter, OR = 5.2 > 5 kg/day dry matter, OR = 7.1) relative to feeding no concentrate; and vaccination with anthrax vaccine during the study, OR = 1.2 relative to no vaccination. Feeding a whole grain with or without other concentrate components reduced risk, OR = 0.6, relative to feeding no whole grain. Results of the study suggest that diet and changes in diet and aging are important risks for colic in a population of horses on farms.

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THE ASSESSMENT OF CATTLE'S WELFARE IN HOUSEHOLD UNITS FROM RUCAR – BRAN AREA

Mitranescu, E.[1], Furnaris, F.[1], Tâpâloâga, D.[1], Simion, V.[2] and Togan, C.[3]

[1] University of Agronomical Sciences and Veterinary Medicine Bucharest, Faculty of Veterinary Medicine; [2] University Spiru Haret Bucharest, Faculty of Veterinary Medicine; [3] National Sanitary Veterinary and Food Safety Authority

SUMMARY

The researches aimed the cattle welfare assessment in household units from Rucar-Bran area. There were observed 28 animals in 10 shelters, being applied the Austrian ANI 35L system.

The parameters, gathered in 5 areas of influence – locomotion, social interaction, flooring, light and air, stockmanship – were scored by measurements and direct observation of the herds or by last generation devices. In order to assess integument condition we have used a method suggested by Cook N.B. (2002) and for hooves condition the gait score (Sprecher D., 1997).

The final score was 19,25 points, indicating poor welfare of cattle in household units.

Keywords: assessment, welfare, cattle, ANI 35L, areas of influence

INTRODUCTION

At present, the animal welfare issue means for human society not just a far-reaching target, but more and more a necessity due to the fact that consumers and general public became aware that the animal welfare is essential in food safety, public health, environmental protection and biologic diversity.

The main importance of animal welfare issue is proved by the fact that it concerns many governmental or nongovernmental organizations with political, economical, social, technical and professional profiles, such as: UN, through FAO (Food and Agriculture Organization); World Trade Organization; the European Council; the European Union; OIE – World Organization for Animal Health; Codex Alimentarius; World Veterinary Association; World Society for the Protection of Animals; Eurogroup for animal welfare.

As Romania joined European Union, a radical change of the way of thinking and approaching the above subject is welcomed. The long-term strategy for improving the level of livestock welfare in our country should include: increasing the number of in-field assessments, in order to obtain sufficient data for statistical processing; drafting a national welfare database for collecting all livestock welfare relevant indicators, no matter the rearing system; establishing both standard assessment methods and national acceptable welfare levels for different farm animal species, by agreement between all parties involved; including the acceptable welfare levels in animal protection legislation and enforcing it.

In this context, the present study aims to establish the welfare level of cattle (youngster and diary cows) reared in household units in Rucar-Bran area, collecting welfare data and enlarging the perception of this subject.

MATERIALS AND METHODS

The researches were run during a period of 5 months (July – September 2005 and December – January 2006), on different categories of cattle (youngster and diary cows) housed in 10 shelters from Rucar, Arges county.

The floors of all shelters had in view were divided in 2 areas: a resting area build of wood and a multi-functional area, from concrete (figure 1, A and B). Animals are tied in large stalls at any time during the year, excepting June 1st – September 1st period, when animals are moved on pasture. Feeding and watering are made manually, ventilation is natural, light is exclusively artificial in shelter no. 6 and natural + supplemental in other shelters. Manure collection and evacuation are made manually, except shelter no. 5 which has a manure pit evacuated once a 6 month.

A *B*

Figure 1. Inner views of Rucar cattle shelters

As Romania has not an official numeric system for animal welfare assessment, we have chosen among various European systems (e.g. Austrian system ANI 35, German system ANI 200, English system used in B.W.A.P. etc.) ANI 35L system, with great applicability and rapidity. The method consists in combining as a sole result engineering-based parameters (details concerning shelter architecture and systems) with animal-based parameters (physiological e.g. condition of integuments, condition of hooves, technopaties or ethological e.g. herd structure and management of the young). Parameters are ranged in five areas of influence – locomotion, social interaction, flooring, light and air and stockmanship – being scored either by measurements, anamnesis data or direct observation of the herds, or by investigations with last generation devices as BK 2250 sonometer (for noises level), Dräger Miniwarn gas-meter (for air quality) and UA6 anemometer (for draughts intensity). In order to assess integument condition we used a method suggested by Cook N.B. (2002) and for hooves condition the gait score (Sprecher D., 1997).

RESULTS AND DISCUSSIONS

The final scores for the assessed shelters in household units from Rucar area are shown in the next table.

Table 1. The final scores for the cattle housed in the 10 shelters in household units

Shelter no./cattle category	Scores /area of influence					ANI 35 final scores	Level of welfare
	Locomotion	Social interaction	Flooring	Light and air	Stockman-ship		
1/diary cows	3 points	1,5 points	4,5 points	4,5 points	5,5 points	19 points	Poor
2/youngster 6–12 months	3 points	2,5 points	4,5 points	4,5 points	5,5 points	20 points	Averag e
3/diary cows	3 points	1,5 points	4,5 points	5 points	5,5 points	19,5 points	Poor
4/ youngster 6–12 months	2,5 points	2,5 points	4,5 points	5 points	5,5 points	20 points	Averag e
5/diary cows	3 points	1,5 points	4,5 points	4,5 points	5,5 points	19 points	Poor
6/diary cows	3 points	1,5 points	4,5 points	4,5 points	5,5 points	19 points	Poor
7/diary cows	3 points	1,5 points	4,5 points	4,5 points	5,5 points	19 points	Poor
8/diary cows	3 points	1,5 points	4,5 points	4,5 points	5,5 points	19 points	Poor
9/diary cows	3 points	1,5 points	4,5 points	5 points	5,5 points	19,5 points	Poor
10/diary cows	3 points	1,5 points	4,5 points	4,5 points	5,5 points	19 points	Poor
Average value	**2,96 points**	**1,83 points**	**4,50 points**	**4,64 points**	**5,50 points**	**19,25 points**	**Poor**

As the herds had not an equal number of animals, the scores for each area of influence and the final scores are calculated as dispersed average values, in order to reveal the general status of all animals.

The final score is 19,25 points, value between 16 and 20 points, so the welfare of cattle is poor. Regarding the scores for the areas of influences, the critical values are: 2,96 points of 10,5 points maximum for locomotion and 1,83 points of 10 points maximum for social interactions.

The main animal housing and management deficiencies are the following: the lack of outdoor spaces, the use of tether system, small space allowance and herd structure based on production or age groups.

CONCLUSIONS

1. The level of cattle welfare in household units from Rucar-Bran area must be improved by measures addressing to housing and management critical situations. This implies the using of loose system instead the tether system, increasing shelter usable space, assuring the animal access to paddocks, keeping the sucklers and youngster with cows (natural group structure).
2. The results of the study prove that in some household units in Romania the welfare is poor, animal rearing still being archaic, without a scientific base. The cause is that the rural population does not understand that welfare is a necessity, both ethical and economical.

3. The present study also demonstrates that a numeric system of welfare assessment is perfectly applicable to in-field conditions of Romania, such a national system should be drafted in our country too.

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DAILY FEED INTAKE – A PARAMETER FOR ASSESSING THE PIG HEALTH STATUS?

Nathues, H.[1], grosse Beilage, E.[1], grosse Beilage, T.[2], Flerlage, A.[3], Flerlage, H.[3], Wolf, P.[4] and Kamphues, J.[4]

[1] Field Station for Epidemiology, University of Veterinary Medicine Hannover, Foundation, Germany; [2] Vetkon GmbH, Essen/ Oldenburg, Germany; [3] Flerlage Farm Management Essen/ Oldenburg, Germany; [4] Institute of Animal Nutrition, University of Veterinary Medicine Hannover, Foundation

INTRODUCTION

The majority of today's food safety concerns have their origin in the production stages prior to slaughter and processing the so-called "pre-harvest" stages, i.e. mainly the agricultural primary production (Blaha 2005). The inclusion of the primary production (feed and animal production) into the food safety system is the so-called "stable-to-table concept" as issued in the basic directives "EG 178/2002" and "EG 183/2005" of the EU Commission. The initiation of such a system needs valid parameters which reflect the health status of the pigs during life time. An approach to measure the health status of fatting pigs is the animal treatment index (ATI). This benchmarking of pig herd health is based on the number of treated animals and the number of treatment days per group and on the duration of the fattening period (Blaha et al. 2006). Enormous variation of ATI (Blaha et al. 2006) requires further parameters to assess the health status.

The objective of this study was to evaluate whether the daily feed intake is a valid parameter to assess health status of penned pigs.

MATERIAL AND METHODS

In a fattening herd keeping 680 pigs in 3 units with a sensor processing feeding technique the daily feed intake of the pigs was documented for each valve separately (tab. 1).

Table 1. Housing of pigs in different units

Unit	Fattening period	Pigs (n)	Valves (n)	Pigs per valve (n)
1	02. May 06 – 23. Sept. 06	198	4	50
2	17. May 06 – 21. Sept. 06	300	4	75
3	17. May 06 – 23. Sept. 06	176	8	22

The lack of memory capacity in feeding computers for the storage of data sets from the whole fattening period required a handwritten documentation. The documentation also included information about clinical signs, antibiotic treatment (individual and/or group) and mortality.
The association between daily feed intake and clinical signs, antibiotic treatment and mortality was investigated using statistical standard procedures (SAS® 9.1, SAS Inc., Cary, NC, USA).

RESULTS

The information of one batch per unit, which has been investigated up today, is presented in tab. 2. Differences in health parameters like treatment days and mortality are negatively correlated with average daily gain and the maximum duration of fattening period.

Table 2. Production parameters for the first batches in unit 1 to 3

Unit	Average daily gain (g/day)	Maximum duration of fattening (days)	Extended fattening period* (%)	Group treatments (days)	Mortality (%)
1	718.4	130	19.2	30	3.0
2	695.3	142	15.4	37	3.3
3	717.0	130	10.2	21	1.7

* Light weighted pigs were kept for an extended period in a separate unit

The daily feed intake for each batch is highly variable and influenced by the feeding scheme as well as the health status (fig. 1 to 3). During the first weeks of the fattening period, pigs were fed *ad libitum* and feed intake was above 100%. A decrease of feed intake for one day was observed in unit 1 and 3 as a result of technical failure. Feed was restricted at least after 10 weeks to avoid excessive fat content in the carcass. One day of decrease in feed intake in the end of fattening was due to the first shipping of slaughter pigs. Further periods of reduced feed intake could be correlated with clinical disease, e.g. coughing from day 109 to 118 in unit 3.

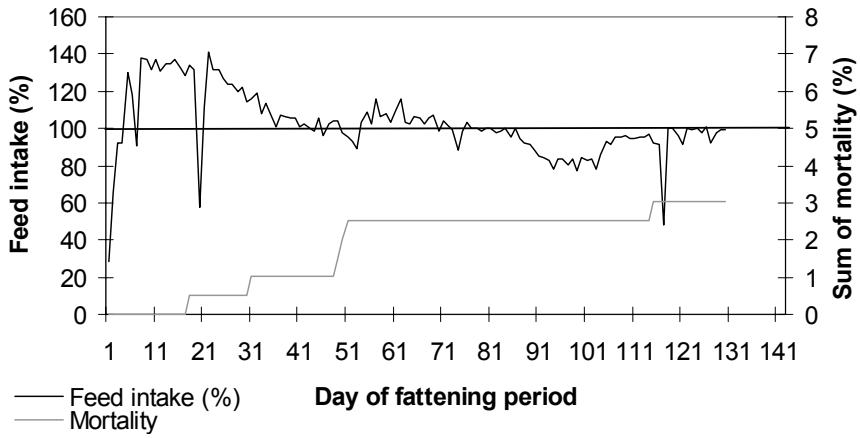

Figure 1. Daily feed intake and mortality for the first batch in unit 1

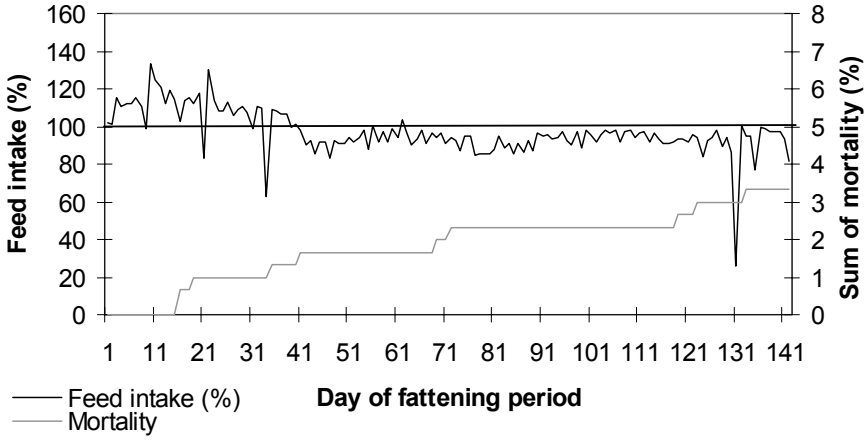

Figure 2. Daily feed intake and mortality for the first batch in unit 2

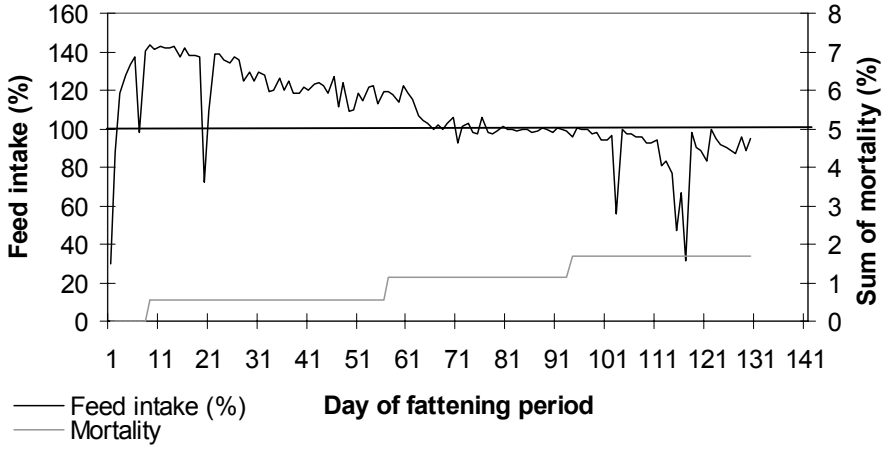

Figure 3. Daily feed intake and mortality for the first batch in unit 3

The study is not completed with this data and further batches to be kept during the winter period will be presented.

CONCLUSION

The cautious interpretation of the results of these three batches of the daily feed intake may be a suitable and reliable tool for measuring the health status of penned pigs. Combined with further parameters like ATI, average daily gain and mortality, the daily feed intake might be an additional type of information to complete the assessment of the pig health status during primary production.

Furthermore, the daily feed intake serves as valuable information for the attending veterinarian to follow up the course of several infectious diseases and control the efficiency of group treatments. In both cases it should be taken into account that feed intake might be influenced by other factors. Reduction in feed intake could also be caused by undesirable compounds, feed deterioration as well as environmental factors (Kamphues 2002).

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"BIOAGRO": A PORTAL THAT AIMS TO THE PROMOTION AND IMPROVEMENT OF ORGANIC AGRICULTURE IN EUROPEAN UNION

Xylrouri–Fragkiadaki, E.[1], Hadjigeorgiou, I.[2], Poulopoulou, I.[1], Paparadi, A.[1], Patrikakis, Ch. Z.[3] and Sideridis, A.B. [3]

Agricultural University of Athens, [1] Dept of Anatomy and Physiology of Farm Animals, [2] Dept. of Animal Nutrition of Farm Animals, Faculty of Animal Science, and [3] Informatics Laboratory, Iera Odos 75, 118 55 Athens, Greece

ABSTRACT

Global community has recently shown a significant interest in environmental conservation in order to assure food safety and quality. Organic Agriculture (OA) as an alternative approach to the conventional intensive farming responds to these demands and has significantly been developed in many European countries and elsewhere. Organic Animal Farming (OAF) systems and the related grassland farming have also gained a significant importance for these systems.

However, ten years after the application of organic agriculture, a combination of problems (lack of technical knowledge and information, reliable system for promotion of organic products) are present. In order for the organic agriculture to continue its growth, solutions to these problems have to be found. Through this frame, the use of Internet can give solutions to the problems listed above.

The aim of this study is the presentation of a reliable service platform, that can operate as the major information source for people related to organic agriculture (veterinarians, agronomists, farmers, food enterprises, consumers etc) in European Union, and in parallel provide e-services regarding OA. The platform is designed and implemented according to the European Union programme e-Content 11293 "Bio@gro". [2]The countries participating in this programme are Greece, Germany, Romania and Cyprus. The content of the portal is given in English and in the four languages of the participant countries, thus the portal can be accessible by a vast number of users. The information provided through the platform is taken from all possible sources (e.g. Ministries, Research Institutes and Organisations) that deal with OA in general. In particular the aim of Bio@gro as regards access to information is the presentation of reliable information (legislation, practice guides etc) and services for the promotion and support of organic agriculture. However, Bio@gro is more than that. It is a comprehensive e-services platform, covering: a) E-commerce b) E-learning c) Digital Library d) Calendar of events related to organic agriculture e) The option of exchanging opinions.

METHODOLOGY

The role of the Bio@gro project is to provide all the interested actors with OA information. This can only be achieved by providing multilingual (and furthermore, multicultural) content. Hence,

[2] More information about e-Content 11923 project and the use of BIO@GRO portal can be accessed through web address http://www.bioagro.gr.

all Bio@gro content is available not only in English but also in each project partner's language (Greek, German and Romanian) with the only exception being the content regarding national legislation, which is provided in English and the corresponding language of the countries participating in this project.

The definition of the content categories, is in line with the methodology followed during the analysis of the user requirements. More specifically, a multi-disciplinary group of content experts has been allocated to identify possible content categories, as well as to assess the results.

Content categories of Bio@gro Web portal

The need for up-to-date information about the OA sector, including events from all around the world and legislative, agricultural, scientific and economic developments, is what has driven the categorization of content for the Bio@gro portal. Using information from governmental (e.g. Ministries of Agriculture, AGROCERT) and non-governmental organisations (e.g. IFOAM, certification bodies) of each country, the daily press, scientific journals, on-line scientific associations (e.g. European Association for animal production – EAAP), educational institutions, processors, traders and consumers' associations, various OA related websites, as well as *de novo* content produced by Bio@gro itself, the portal comprises one of the most comprehensive sources of information available.

The information is classified in eight main categories (see Figure 1).

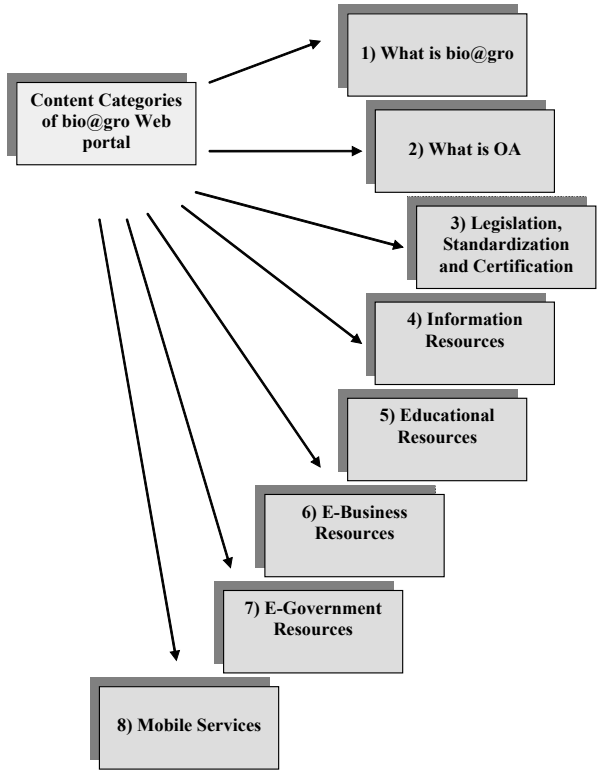

Figure 1. List of Content Categories of Bio@gro Web portal

A detailed description of the categories of the portal is given below.

What is Bio@gro

Bio@gro project proposal can contribute to the integrated development of the OA sector throughout Europe by offering improved conditions and new opportunities not only to organic farmers' agribusinesses, but also to European citizens. The overall objective of this proposal is to create a critical mass of OA material and mechanisms, including OA actors from all links of the value chain, and to develop an e-services system as a single point of access for OA information and business opportunities.

What is OA

This section includes the core definitions for each OA actor, the principles of OA, and glossary of terms, as presented by various validated OA sources, such as non-profit organisations, foundations, research institutes, universities and scientific journals.

A number of selected and representative definitions of OA will be provided, based on those given by organisations or individuals whose expert knowledge has been validated through their active and long term involvement in the OA field.

Basic principles of OA are presented, based on those determined by the International Federation of Organic Agricultural Movements (IFOAM), as well as threats arising from conventional agriculture and differences between organic and conventional agriculture.

A list of terms along with a brief explanation of their meaning is provided in this part of the content with special emphasis given to terms that help in defining and differentiating OA practices from the conventional ones. This information originates from official, validated sources, such as EC information centres and educational institutes.

Legislation, Standardisation and Certification

An important part of the project is the legislation and certification that governs the field of OA. The Council Regulation (EEC) No 2092/91 on organic production of agricultural products and the national OA legislation of each partner country is described in this section, as well as the legislation and certification of other major European and third countries (such as UK and USA, Canada, Australia, Japan).

Information resources

The main categories of the available information resources are presented in the following:

- News about OA, announcements, events (such as eco-festivals, conferences), OA events calendar service.
- Digital library with OA reports, studies, papers, legislative documents.
- Directories of OA-related links, such as:
  - European and National OA Initiatives,
  - Related agencies, such as certification bodies and monitoring organisations,
  - Useful links to other related web sites.

Educational Resources

The Bio@gro project addresses the needs of a variety of users ranging from simple individuals who are not familiar with OA and simply require general information about OA, and professionals engaged in this area (farmers, processors and traders) and need more specific information. This project provides educational resources such as e-learning courses and best

practice guides in order to help these different categories of users fulfil their requirements and provide integrated services. This section also includes a catalogue of other online educational resources and a number of frequently asked questions (FAQs) related to OA.

E-Business Resources

A description of the resources covered by this subsection is described in the following:

Business outlook:

- State support, subsidies, organic advisory services and programs and related services, such as taxation related to OA (European and National).
- Market reports, market information and trends, OA product price reports and status, etc.

Online shops and markets with OA products directory:

- Advertising services, where interested parties can promote their enterprises and/or products by placing a logo or e-mail service.
- Presentation of offered (products and services in a common pool of all European OA enterprises (e.g. by a link from the European list to the specific homepage).
- Potential customers' support services, such as OA producers and markets recommendation services (provides support for sales of products and services which comprise potential purchases).

List of labels of Organic Products:

- Information about labelling of Organic Products for each Member State of the European Union or imports from third countries.

Directories of suppliers, traders, farmers:

- Information for contact making with suppliers, i.e. support for the purchase preparation of necessary products and services.
- Bio@gro registered members catalogue (registered OA producers / processors / traders / farmers / researchers/ /consulting and controlling bodies)

E-government resources

In this section, a brief description of the e-government resources available through the Bio@gro portal is provided. In details, this section includes:

- A directory of Governmental Organisations and agencies providing information for OA actors.
- A directory of online services offered by Governmental Organisations and agencies.

Mobile services

Mobile services are also an important part of the Bio@gro portal. These are based on the exchange of SMS messages that is used for the transmission of alerts and notifications regarding:

- OA news.
- Bio@gro portal updates.
- Weather forecasts.
- Crop protection alerts.

Structure of Customers / Users / Stakeholders Groups

Considering that one of the important goals in trying to establish Bio@gro in the field OA was to analyse the current structure of the Market in the field of OA, we started with a shortlist of

stakeholder classes and groups with similar or related functions and interests. According to the previous investigations carried out by the Bio@gro team, the following user classes and groups (stakeholder classes) were identified:

- Business class: Farmers, Processors, Traders, Consultants and advisers.
- Non-profit class (information and communication): Administration, Associations and organisations (NGO's), Control Bodies, Researchers and Consultants, advisers.
- Consumer's class: Consumers, citizens.

This classification is not static. Many users cannot be clearly assigned to a single specific group, or there are operators belonging simultaneously to different groups (farmers and processors, farmers and traders, traders and processors).

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D –
FREE COMMUNICATIONS

ORAL PRESENTATIONS

FOUNDATION OF A EUROPEAN COLLEGE OF SMALL RUMINANT HEALTH MANAGEMENT [ECSRHM]

Fthenakis, G.C.

Veterinary Faculty, University of Thessaly, 43100 Karditsa, Greece;
Organising Committee ECSRHM; e-mail: gcf@vet.uth.gr

Following an organisation period of two years, the European Board of Veterinary Specialization approved the establishment of the European College of Small Ruminant Health Management [ECSRHM]. The speciality refers to: a) Husbandry, internal medicine, surgery, obstetrics and reproduction, as applied to epidemiology, pathogenesis, diagnosis, therapy, control of diseases of small ruminants (sheep, goats); b) Individual patients or flocks/herds; c) Quality and safety of products from small ruminants; d) Control of transmitting zoonoses.

Objectives of the College are: a) Acting to qualify veterinarians as specialists in small ruminant health and production; b) Developing graduate teaching programmes in small ruminant health and production; c) Developing and supervising continuing education programmes; d) Encouraging its members to pursue original scientific investigations and to contribute to the relevant literature; e) Defining and describing the speciality; f) Supervising the professional activities of its members; g) Promoting collaboration.

Members of the College will be designated as “Dipl. ECSRHM” and will be appointed either as de facto Diplomates (up to April 2012) upon experience and academic achievements or alternatively by examination after following a defined period of training (residency).

A BRIEF HISTORY OF THE SPIRIT OF ANIMAL HYGIENE

Hartung, J.[1] and Schäffer, J.[2]

[1] Institute of Animal Hygiene, Animal Welfare and Behaviour of Farm Animals;
[2] Institute of Veterinary History, University of Veterinary Medicine Hannover, Germany

SUMMARY

The term "animal hygiene" is a regular component of veterinary science and education since the beginning of the 19th century focussing on health care and the investigation of the origin of animal diseases although "Hygiea" goes back to the early roots of civilisation and the myths of ancient Greece. The principle is prevention of diseases as against to cure by creating a pleasant, harmonious and stress-free living environment for both a single animal and a herd. Bio-security is only a part of this concept. The scientific goals of the ISAH should continue to develop the necessary methodology for precaution and prevention, health care and protection of all domestic animals and minimise spread of food borne zoonosis.

Keywords: animal hygiene, veterinary history, biosecurity, prevention, zoonosis

INTRODUCTION

Klimmer wrote 1914: "The preservation of health (Gesundheitspflege) of farm animals is that part of veterinary science which helps us to recognise the causes of animal diseases and teaches us how to prevent factors that cause disease by improving disease resistance without neglecting the economic purpose of livestock production." Animal Hygiene is Preventive Medicine, it stands opposite of Curing Medicine. The overall aim of animal hygiene is to keep animals, farm animals and companion animals, healthy and protect them from all factors that can impair their health, well-being and production. It is a holistic approach preventing disease and discomfort instead of curing. This approach is not limited to typical food delivering animals such as cattle, sheep and pigs, it applies also to domestic and companion animals like horses, ferrets or falcons.

The term "animal hygiene" appears in German medical/veterinary text books at the beginning of the 19th century. Hygiene became a regular component of the veterinary science and education focussing on health care and the investigation of the origin of animal diseases. Personalities like Klimmer influenced the curriculum at veterinary universities and in practice by introducing "hygiene as preventive medicine" since the beginning of the last century. The idea of "hygiene" is very much older and goes back to the early roots of civilisation.

ROOTS OF ANIMAL HYGIENE

The idea of curing instead of therapy goes back to the ancient times of Greek mythology. The importance of hygiene in medicine of those days is demonstrated by the fact that "Hygieia" was in the rank of a goddess. Hygieia was the daughter of the goddesses Asklepios and Epione. Asklepios was the son of Apollon and Coronis. He had been a student of the centaur Cheiron and was educated to heal diseases. Epione's task was to ease pain, to soothe and comfort patients. Both recognised very soon that neither his skills to heal nor her abilities to care and comfort were able to avoid disease and suffering of their patients. Therefore they engendered her child Hygieia who should prevent the initiation of diseases and all forms of suffering by creating a pleasant, harmonious and stress-free living environment, emphasising the principle of hygiene that prevention is the first choice and better than to cure.

These preventive principles are found in the definition of modern animal hygiene again which is intended to protect the health and well-being of a single animal as well as of a herd by providing animal-suited keeping and feeding systems, hindering the invasion of infectious agents from outside and the spread within the herd, promote well-being by gentle handling, strengthen the animal's immune system and resistance by observing their (behavioural) needs and reduce application of drugs to the absolute necessary minimum. This clearly demonstrates that the recently introduced term "bio-security" is important but can cover only a part of the concept of animal hygiene. The scientific goals of the International Society of Animal Hygiene should continue to develop the principles of precaution and prevention, health care and protection for all domestic animals, food producing animals as well as pets and horses. This is not only for a better health and well-being of the animals; it also improves production of wholesome food and helps to protect animal owners and consumers against zoonotic diseases.

DEVELOPMENT OF ANIMAL HYGIENE OVER THE CENTURIES

The first practical measures to support health of people and prevent disease are going back to the early ancients. In Babylon, about 4500 BC, fresh water supply and waste water were separated (Codex Hammurabi). The Egyptians and the Jews had hygienic prescriptions for food, clothing and cleaning to prevent leprosy and other diseases. The Egyptians had already some knowledge about endoparasites. They branded cattle for identification of ownership before the grazing season. Animal houses for cattle are reported from about 1400 BC in Egypt. In some drawings of such stalls drains for urine and liquid manure are indicated.

In Athens and Sparta (Greece), public health care was part of legislation (Lykurg 1800 BC). Plato and Aristoteles asked for sanitary police and initiated the drainage of marshlands close to the city and promoted controlled drinking water supply and public baths. And naturally the books of Hippocrates on life style, air, water and environment were fundamental contributions to the development of life saving hygiene.

Important principles of animal hygiene are precisely described by Aristoteles (about 384 to 322 BC) for horses, cattle, pig and dogs in his script: "Natural history of animals". Xenophon (426 to about 355 BC) recommends cleaning and treatment of animals outside the animal house. Head, mane and tail should be frequently curry-combed and cleaned using a sufficient number of towels. Stables should be paved and littered (dry leaves and straw) and manure should be removed daily. Horses should be loosely tethered by a rope around the neck to the trough and no longer by tethering the legs. Drawings of that time show e.g. a stable with stands for 6 horses.

Slits in the wall in front of the horses served probably as openings for the halter-strap. Rectangular holes in the wall above the animals may have been part of a ventilation system. Many Greek and Roman stables had outdoor sand areas (paddocks) directly connected to the stall where the horses could rest, groom and roll over. It was also recommended to feed only hay of good quality without sand and visible fungi growth, water should be offered after eating and animals which sweat or are exhausted should first calm down before water is given. Flowing water was preferred to standing water supplies.

Lucius Junius Moderatus Columella (first century AC) recommends regular inspections of sheep herds to recognise early epizootics such as anthrax. All diseased animals should be separated and fallen animals buried in the ground at certain remote places (Vegetius Renatus 4th Century AC). Vegetius Renatus gave also advice for housing including floor, feeding troughs, racks, light and ventilation.

With the decay of the Roman Empire much of the hygienic knowledge was lost; water supply and public hygiene became poor. Later, in the 12th century, Pope Zacharias found it necessary to issue a regulation which forbade eating meat from diseased animals. In the town of Augsburg public slaughter houses were founded by the magistrate and those parts of diseased animals which seemed to be eatable yet had to be sold under special labelling. From 1404 such meat was sold through special shops (Freibank, Finnbank, "Trichinella meat shop") only (example town of Wimpfen). However, it did not help much. During the devastating epidemics in the middle age millions of people (estimated 26 million between 1346 and 1353) and animals were killed.

It was only in the 18th century that the crucial importance of hygiene was re-discovered again. Between 1711 and 1717 strict hygienic legislation was introduced in the Kingdom of Prussia and by the Duke of Saxony to prevent the spread of infectious epidemic diseases among animals. These were probably the first general public veterinary health rules in Europe. Farmers and inspectors were bound to report notifiable diseases, built up quarantine sections, kill diseased animals and dispose carcases safely (usually by burying or burning) at specified places. Important are also the contributions of Lavoisier (1768) to the hygiene of housing and accommodation, drinking water supply, waste removal by canalisation, ventilation, air hygiene etc. Science and hygiene started to grow during the second half of the 18th century. That is also the time when the first Veterinary Schools were founded: Lyon (1772), Alfort (1776) and Hannover (1778).

THE NEW AGE

With the discovery of the vaccination technique (Jenner 1796) a new area started. Vaccination gave the opportunity to protect the animals of a herd and the herds in a region against specific infectious agents. A bundle of hygienic measures around the animal supported the resulting individual immunity by removing as good as possible all stressing factors from the animal's environment. Hygienic measures were also taken to prevent the spread of infectious agents between animals on the same farm and between herds of different farms by avoiding animal and human traffic (direct contact) between farms as well as air transmission or living vectors like rats, mice and flies. By this combination of improved resistance against infectious agents and strict hygienic biosecurity measures many animal epidemics such as Rinderpest, tuberculosis, brucellosis, small pocks of sheep, malleus, cholera of fowl, rabies and others could be eradicated stepwise or massively reduced in Europe during the 19th and 20th century.

PRESENT SITUATION

However, recent outbreaks of foot and mouth disease in Britain, the swine fever epidemic in the north of Germany in the early nineties of the last century and the actual concerns about Avian Influenza Virus which has a zoonotic potential demonstrate how difficult and fragile the system of disease control still is under the terms of modern intensive, specialised and regionally concentrated animal production. Another thread arises from the multi-factorial infectious diseases such as enzootic pneumonia, infectious bronchitis or COPD and also from behavioural disorders such as feather pecking and cannibalism in poultry and swine which both develop preferentially under intensive keeping conditions. These diseases are not caused by a specific infectious agent but by a number of environmental factors such as inadequate housing, poor air quality, bad handling and insufficient quarantine and biosecurity measures (cleaning and disinfecting). Striking examples are rates of pneumonia as high as 30% in slaughter pigs and continuously high salmonella contamination rates in poultry.

CONCLUDING REMARKS

A sustainable control of animal and zoonotic diseases needs beside vaccination applied hygiene concepts. These have to be based on a thorough understanding of:

(1) both type and quantity of the environmental factors which influence health, well-being and performance of animals,
(2) the keeping and housing systems, litter, bedding, ventilation, air quality, manure removal, storage and land application, feeding practices,
(3) the aerial transmission of gases, dust, toxins and micro-organisms to be able to create “safe distances” between farms and between farms and residential areas,
(4) safe manure handling and disposal without posing harm to air, soil and water,
(5) biosecurity measures including cleaning and disinfecting,
(6) the animal’s nature and behaviour that staff can handle the animals properly,
(7) the stress animals may suffer in housing situations, during transport and slaughter in order to protect the animals’ well-being and avoid transmission of zoonotic agents into the food chain.

Animal hygiene, the daughter of Asklepios and Epione, is a difficult child. However, taking care of her and working towards her principles of prevention while using many different scientific disciplines is so interesting and promising that it is important to continue the development of the analytical tools and practical applications for the sake of the animals and man. We should not fall back to the middle age when the holistic view and the high standard of knowledge of the ancient days were lost. When we all are prepared to support Hygieia, animal hygiene will have a great future.

WELFOOD VIRTUAL CAMPUS FOR ANIMAL WELFARE – ENVIRONMENT – FOOD QUALITY AND SAFETY STUDIES

Szücs, E.[1], Geers, R.[2], Praks, J.[3], Sossidou, E.[4], Jezierski, T.[5] and Poikalainen, V.[3]

[1] Szent István University, Faculty for Agricultural and Environmental Sciences, Gödöllő, Hungary; [2] Catholic University, Zootechnical Centre R&D, Leuven, Belgium; [3] Estonian University of Life Sciences, Institute of Veterinary Medicine and Animal Sciences, Tartu, Estonia; [4] National Agricultural Research Foundation Animal Research Institute, Giannitsa Greece; [5] Polish Academy of Sciences, Institute of Genetics and Animal Breeding, Jastrzebiec

ABSTRACT

The WELFOOD Virtual Campus has been developed as a vocational training program on animal welfare – environment- food quality and safety interactions with a global look at all issues related to animal welfare in farms, during transport and slaughter, including processing of food and traceability of quality information implications. The reason was that training on animal welfare as such with regard to recent research results in relation to both environmental interactions, and food quality and safety, are becoming in the limelight of consumers and enhancing societal awareness. Modules are assigned into three Courses: (1) Animal welfare, (2) Environmental impacts on and of animals, and (3) Food quality and safety. The areas cover different topics and lessons. The basic materials are uploaded to the Project Homepage *(http://www.welfood.szie.hu)* and upgraded in English as lingua franca and languages of the Partner countries (Estonian, Flemish, Greek, Hungarian and Polish). In depth background materials on the topics are available in English. Access to further studies can be consulted through links to glossary, relevant literature and websites. For better understanding and demonstration video clips provide assistance the learners. The performance of students is evaluated through computer random generated quizzes, and assays/case studies depending on education levels (vocational training, BSc, MSc, and PhD). Impetus for applying e-learning was given to this development due to the high didactic value of ICT. E-learning can be defined as delivery of a learning, training or education program by electronic means. E-learning involves the use of a computer or electronic device in some way to provide training, educational or learning material *(Stockley, 2003)* which can also use a variety of equipments in online training or education, such as even CD-ROMs and DVDs. This web based system can be managed in a so called Moodle (Modular Object-Oriented Dynamic Learning Environment) program (*Vágvölgyi, 200)*. Model is a software package for producing internet-based courses and web sites *(http://docs.moodle.org; http://www.moodle.org; http://www.moodle.com)* which is provided freely as an Open Source software package for course management system (CMS). The system is designed using sound pedagogical principles to help educators create effective online learning communities. The most important advantage of ICT assisted education system is the world wide and at any time accessibility in virtual environment.
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INTRODUCTION

The greatness of a nation and its moral progress can be judged by the way its animals are treated" as Mahatma Gandhi put it (cit.: *Appleby and Hughes, 1999).* In the past and present people have always been concerned for animal welfare, but what is animal welfare, and what is good treatment? *Appleby (1996)* formulated the definition as "The state of well-being brought about by meeting their physical, environmental, nutritional, behavioural and social needs of the animal or groups of animals under the, supervision or influence of people." In *Fraser's (1989)* view "well-being" refers to endogenous states of being within an animal and "welfare" to human interventions designed to promote well-being. In *Hughes's word (1976)* welfare is "a state of complete mental and physical health, where the animal is in harmony with its environment", but it may be a subject of change. "Welfare can vary between very poor and very good. In order to use the concept of welfare in a scientific way it is necessary to specify the level of an animal's welfare and not simply to reserve the word to indicate that the animal has, or does not have, problems" (*Broom and Johnson, 1993).* However what does "problems" mean? History, background and philosophy are dealing with "issues" and "problems" in which animals may suffer. To measure well-being we speak about assessment, to ameliorate suffering about solutions and about how to solve them in practice about implementation *(Appleby and Hughes, 1999).*

In a recent, comprehensive presentation discussed central moral issues involved in the treatment of animals in agriculture and introduces the major ethical concepts and principles that pertain to animal bioethics *(Pascalev, 2004).* It explores critically the concept of animal rights, animal suffering, animal welfare, and the moral values behind such movements as vegetarianism and animal liberation. Special attention is given to the issue of animal welfare in light of the latest advances in biotechnology such as cloning, genetic engineering and xenotransplantation. Some of the addressed questions are: What are the main ethical challenges that animal agriculture faces today? Is it moral to genetically engineer farm animals and can the need for greater productivity justify the genetic modification of such animals? Should we change the natural capacities of animals e.g., to reduce their ability to feel pain and increase their resistance to disease? What is the moral status of animals with human genes or genes from other animal species? What is involved in respecting animals?

Ever since there have been raised quite a lot of open questions to be answered. Consumers require healthy, safe and high quality food. Food production systems are tending towards those which are safe, sustainable, environment- and welfare- friendly, and which have low requirements for inputs. Interest of public has recently focussed mainly on three main areas in this issue such as animal welfare; environmental impacts on and of animals; and last but not least high quality and safety of foods. For this reason, significance of knowledge on quality assurance has been increasing in all phases of food production of animal origin. This novel approach of the topic requires special skills in sectors involved such as animal husbandry and food industry. Following the principle "from fork-to-farm approach" in foods of animal origin, i.e. traceability, transparency and labelling, research on production methods should aim to meet the consumer's requirements. Recent experiences and requests from commercial practice reveal expanding demand for experts who are skilled in the subject matter. Thus, knowledge on animal welfare x environment x food quality and safety interaction is needed for the development of competitive technologies with a global look at all issues related to animal welfare in farms, housing, processing and food safety of commercialised products. In the WELFOOD project multidisciplinary approach is applied for the transfer of recent scientific findings and knowledge on the specific area of this pilot project. Driving force for the application of e-Learning will be

dedicated to apply ICT having high didactic and added value and rapid transfer of knowledge in a fast and efficient way.

WELFOOD addresses objectives such as improvement and competencies of the skills in vocational training to promote employability and facilitate integration and reintegration in terms of capabilities and knowledge needed for improved technologies in animal husbandry and food industry. Emphasis is laid on skills in food quality assurance issues related to animal welfare x environment x food quality interactions required by public perception due to their role in food safety and security as well as ethical considerations. Significance and novelty of this area are in line with the recent developments in the EU and the rest of the world.

STRUCTURE AND DESCRIPTION OF COURSES

Teaching materials of WELFOOD are structured into three Courses:

1. Animal welfare
   - Ethical views concerning how to treat animals and their justifications
   - Definitions of animal welfare in domestic animals
   - Welfare assessment of production systems
   - Improving welfare status of animals in different phases of production chain
   - Animal welfare and preslaughter handling

Thus, the first course of the WELFOOD project focuses on the welfare of animals. The aims of this course are first of all to talk about the ethical views concerning how to treat animals. Secondly the definitions of animal welfare in domestic animals are described. The third objective is to speak about the assessment of welfare in different production systems. The ways to improve the welfare status of animals in different phases of the production chain and the effect of transport en preslaughter handling on welfare are also aims of this course.

2. Environmental impacts on and of animals
   - Challenge of artificial environments to domestic animals
   - Nutrient efficiency, direct and indirect emissions, manure handling and processing

This second course focuses on main aspects of environmental impact on and of animals. Animal husbandry and production chain of food of animal origin are characterized by complex interrelations with environment. Animal husbandry creates a burden to the environment e.g. by pollution with manure and on the other hand the quality of animal products depends on the quality of environment in which the animals are maintained and production takes place. Today's environment becomes more and more artificial and may have undesirable impact on animals. The quality of animal products and animal welfare may be influenced in a positive and desirable way by changes of the environment. This course is divided into two areas related to: (1) impact of environment (mainly feeding) on animals, and (2) impact of animals on environment (mainly manure handling and processing). Special attention is given to the most important animal products as milk, beef and pork and to possibilities to improve the quality of these products by modifications of feeding and animal maintenance systems. In relation to the growing interest of consumers for local food products of special taste, some materials are devoted to such local products at least in some countries.

3. Food quality and safety
   - On-farm risk analysis
   - HACCP at farm level
   - Food and other products deriving from GMOs
   - Animal welfare implications of farm assurance schemes
   - Traceability and transparency
   - Animal welfare-environment-food quality interactions : production consequences

This course focuses in the farm animal welfare quality specifications and standards and aims to promote food quality and safety in animal welfare-environment-food quality interaction studies though upgraded e-Learning. The course aims to support new knowledge and skills on quality assurance in all phases of sustainable food production of animal origin by further improvement of curricula in vocational training. In this module the candidates will first get an introduction to on-farm risk analysis, HACCP at farm level, GMOs products, they will study animal welfare implications of farm assurance schemes and the 'fork to farm' approach and then, they will explore the role of the quality assurance schemes at initial stages of the supply chain, from the point of production to slaughter. Basic specific concepts, ethics and legal aspects will be discussed and detailed examples will refer to human attitudes to farm animals, welfare and conservation. Further, the candidates themselves will test and study different systems and assurance schemes used in the livestock sector, through the use of scenarios, role-play and different collaborative tools in relevant subject contexts.

COURSE E-LEARNING ACTIVITIES

For participating into the WELFOOD Course, students are urged to enrol on the website *http://welfood.szie.hu*.

The WELFOOD Educational Platform is based onto Model Open Source E-Learning platform *(http://docs.moodle.org; http://www.moodle.org; http://www.moodle.com)*. Logging into the platform, students are introduced into the WELFOOD project and gets an overall idea of the structure of the Educational Material organized into the three major e-Courses. The front page of the website gives a general introduction to the WELFOOD project and an overview of the main content of the different courses included in the WELFOOD project. The student can choose between three different courses. Courses consist of a number of lessons. For every lesson there is an Internet-lesson and the full material is available in PDF-format. As a rule, the Internet lessons are destined for users who consider themselves as beginners and full material is destined rather for higher education, however, some full materials that are labelled as for vocational training are suitable for beginners.

For each topic there is a practice quiz, where students can test their skills without being graded. In the practice quiz students can submit each question separately to check their answer. The practice quiz can be saved without submitting so the student can continue with the quiz later. When the quiz is submitted the correct answers are shown. Practice quizzes can be applied several times. At the end of a course there is a final quiz. Students have two attempts to do this final quiz and this quiz is graded.

In the courses there is a link to the main Glossary where specific terms can be browsed alphabetically. There are different ways to communicate with other student(s) and with teacher(s).

There are forums for asynchronous communication. By adding a new discussion topic on a forum students can post their question which can be answered by other students or teachers on another moment. A student can also check the existing forum for other information. In the chat-rooms for synchronous communication student can communicate in real time with other students and teachers.

On the calendar students can check for upcoming events. It is also possible to check the grades.

A student enrolled in the course should follow the lessons. If he/she wants to learn more about the subject, he/she can read the full material added to each lesson or can use the different links whit the internet inside the lessons. To see pictures, tables or other available information there are links inside the presentation. To test their skills student can use the practice quizzes available for every topic. If student have questions they can ask them to teachers and other students by using forums and chat-room.

The WELFOOD web based system can be managed in a so called Model (Modular Object-Oriented Dynamic Learning Environment) program (*Vágvölgyi, 2004)*. Model is a software package for producing internet-based courses and web sites *(http://docs.moodle.org; http://www.moodle.org; http://www.moodle.com)* which is provided freely as an Open Source software package for course management system (CMS). The system is designed using sound pedagogical principles to help educators create effective online learning communities. The most important advantage of ICT assisted education system is the world wide and at any time accessibility in virtual environment.

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"ALL IN – ALL OUT" FINISHING UNITS FOR DAIRY BEEF PRODUCTION

Tuovinen, V.[1], Saarikivi, M. [1], Hartikainen, K. [1], Kauppinen, R. [1], Herva, T.[2], Manninen, U.[3] and Kivinen, T.[4]

[1] Savonia University of Applied Sciences; [2] A-Farmers Ltd., [3] FarmiMalli Ltd., [4] MTT Agrifood Research Finland

SUMMARY

"All in – All out" is a production system, where animals are moved into and out of facilities in distinct groups. Facilities are cleaned and disinfected thoroughly between groups of animals.

Ten days to three weeks old dairy calves are transported to the "all in – all out" calf rearing units. The calves are transported for a second time to the finishing units at the age of 5 – 6 months. They are slaughtered 10 – 14 months later.

The existing finishing units operate on a continuous basis. The "all in – all out" principle in the finishing units is modelled in a pilot project.

Keywords: "all in – all out", calves, finishing, beef, disease prevention, 3D modelling

AIMING FOR "ALL IN – ALL OUT" BEEF FINISHING

"All in – All out" is a production system, where animals are moved into and out of facilities in distinct groups. The aim is to reduce the spread of disease by preventing the mixing of groups. Facilities are cleaned and disinfected thoroughly between groups of animals. The "All in – all out" production method is common in pig and poultry production, but uncommon in cattle production.

Our goal is to introduce the "all in – all out" principle to bovine meat production including the finishing units. The "all in – all out" production is modelled in an EU-funded project run by the Savonia University of Applied Sciences in cooperation with a slaughterhouse company (A–Farmers Ltd.), a company producing 3D farm models (FarmiMalli Ltd), and MTT Agrifood Research Finland. The project ends in December 2007.

BOVINE MEAT PRODUCTION IN FINLAND

About 80% of the annual bovine meat production of 84 million kilograms in Finland originates from dairy breeds. About a quarter of a million cattle are slaughtered each year. Usually, ten days to three weeks old dairy calves are sold to calf rearing units through broker companies owned by slaughterhouses.

An "all-in, all-out" approach is commonly used in calf rearing units. Each batch of calves is treated as a unit from the time of arrival on the farm, until departure at the age of 5–6 months. New animals are not added to the group.

Five to six month old calves are transported to finishing units. They are slaughtered at the age of 16–18 months, when their average slaughter weight is about 330 kilograms.

FINISHING UNITS ARE NEVER EMPTY

Almost all finishing units are never empty, but operate on a continuous basis. Slaughter weighed animals leave and new calves come every month. This means that new diseases come on a monthly basis, because the animal is the ultimate source of an infection. Disease pressure cumulates year by year. Respiratory diseases cause the biggest problems.

Introducing an "all-in, all-out" system would give producers the opportunity to thoroughly clean and disinfect the entire barn before the next group, thus getting rid of the diseases in the previous batch. The producer should not deviate from the "all-in, all-out" system, because retaining some of the calves in the barn will not allow thorough cleaning, and diseases can be more readily transmitted from one group of calves to the next.

The "all in – all out" principle is easy and understandable in theory, but it has not been applied in practice, because it is not as easy in practice as in theory. Farmers need clear models and motivation to apply the principle. The continuous system is reasoned with better use of facilities, monthly money flow, lower price risk and better availability of calves. These are relevant reasons, and must be solved in modelling the "all in – all out" production.

LITERATURE REVIEW

Beef cattle feedlots are the most common way of finishing cattle into marketable beef. Beef feedlots have evolved from small family farm lots into large enterprises that market thousands of finished cattle annually. Farm feedlots in North America and so called "barley-beef "units in the United Kingdom and other European countries still account for a significant part of the feedlot industry. However, the ultimate goal for every finishing unit is to produce marketable beef at the lowest cost and in the shortest time possible (Radostits, 2001, Lechtenberg et al., 1998).

Efficiency of beef production has been improved by new knowledge of nutrition and breeding. It appears that herd health programmes are the only things that can provide significant economic benefits to the feedlot industry in the future.

Diseases are the major cause of economic loss in the feedlot. The impact of clinical and subclinical diseases on productive efficiency and economic returns may be greater than the losses associated with mortality. Infectious diseases of respiratory and alimentary tract are the most common health problems in feedlots. It is a well known fact that mixing animals from different groups or farms increase the risk of infectious disease outbreak and mortality (Radostits, 2001, Maes et al., 2004).

In disease control it is unrealistic to depend on a vaccine, an antibiotic or a single management technique (Radostits, 2001, Lechtenberg et al., 1998). Already the second edition of Herd Health Food Animal Production Medicine (Radostits et al., 1994) recommended adopting the "all in all out" principle to decrease incidence of disease. The third edition of (Radostits, 2001) includes the same recommendations.

The "all in all out" principle is unanimously recommended for beef industry by the veterinary experts, but there is not much information available about it. The principle has been applied in just a few field studies or medical trials. It is not known exactly, how long the empty period should be, nor which diseases can we get rid of with different variations of the "all in – all out" principle.

A longitudinal study of *Escherichia coli* O157 in a finishing unit showed that the source of *E. coli* O 157 was the unit itself, not new animals. Washing procedure and empty period of one day

were not sufficient to destroy *E. coli* O157 (Lahti et al., 2003). This kind of information is very important meeting the requirements for food safety and providing new quality assurance systems (Dagg et al., 2006).

There has also been growing demand from society that farm animals should be kept in ways that take into account the welfare of animals. The "all in all out" supports natural behaviour in groups without excessive stress caused by mixing the groups (Radostits, 2001; Lidfors et al., 2005).

In the pig and poultry industry the "all in – all out" is the most common type of finishing. It is one of the most important management factors in disease control (Radostits, Barnes et al 2000).

The "all in – all out" system is a protective management factor against mortality even in very common infectious disease like *Haemophilus pleuropneumoniae* infection in pigs (Hunneman, 1986). Average daily gain and feed conversion are better in pigs reared in an "all in – all out" system compared with those reared in the continuous system (Radostits, 2001).

In disease eradication programs it is essential to clean the facilities properly to succeed (Heinonen et al, 1999). Using the "all in – all out" system it was possible to raise finishers without *S. Typhimurium* infection despite the fact that the pigs were born in herds with a high level of salmonella infection in the finishing pigs (Dahl et al, 1997). Cleaning the facilities and disinfecting is a protective factor against enteric disease in grower- finishing pigs (Pearce, 1999).

MODELLING "ALL IN – ALL OUT" FOR BEEF PRODUCTION

Seven finishing units were filled in autumn 2006 with a group of calves of the same age. In addition, eight farms agreed to design their new facilities based on an "all in – all out" principle.

Possibilities and benefits of adjusting feeding by the age of the group are monitored. Work load and any practical comments given by the pilot farmers are recorded. Morbidity, mortality, average daily gain, slaughter weight, weight variation, carcass classification and meat inspection findings of the pilot farms are compared with earlier results of those farms and average results of the slaughterhouse. Finally, the total economic debit/credit is calculated. These results are expected from all pilot farms in January 2008.

Modelling of calf supply and transport, slaughter transport, prevention the risk of price variation and possible other aspects are carried out by the slaughterhouse company.

Building models are produced based on the eight pilot buildings. The models named "Finishing farms 2015" are presented three dimensionally (3D) with the new Farm Designer program developed by FarmiMalli Ltd. Building models are designed in cooperation with the building master of FarmiMalli Ltd. and the architect of the MTT Agrifood Research Finland.

The first 3D "all in – all out" models will be presented at the Tartu conference. Any comments of the models are welcome.

The final models will be presented on the web page of the project (www.vasikka.fi) in spring 2008.

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NETWORKED ACADEMIC SOCIETIES IN COLLABORATIVE DEVELOPMENT OF E-LEARNING SOFTWARE FOR VOCATIONAL TRAINING ON THE DOMAIN OF FARM ANIMAL WELFARE-ENVIRONMENT-FOOD QUALITY AND SAFETY

Sossidou, E.N.[1], Szücs, E.[2], Geers, R.[3], Jezierski, T.[4], Praks, J.[5] and Poikalainen, V.[5]

[1] National Agricultural Research Foundation, Animal Research Institute, Giannitsa, Greece, [2] Szent István University, Faculty for Agricultural and Environmental Sciences, Gödöllő Hungary,[3] Catholic University Leuven, Zootechnical Centre R&D, Lovenjoel, Belgium, [4] Polish Academy of Sciences, Institute of Genetics and Animal Breeding, Jastrzebiec, Poland, [5] Estonian University of Life Sciences, Institute of Veterinary Medicine and Animal Sciences, Tartu, Estonia

ABSTRACT

This paper describes the framework and the results derived from WELFOOD Networked Academic Society towards the collaborative development of an e-Learning course on the domain of farm animal welfare, environment and food quality. It has been concluded that developing courses in this way has outstanding benefits: wider knowledge and better e-Learning training skills for each of the members of Networked Academic Society and a higher quality course as compared to similar courses delivered locally by one or only few academics.

Keywords: farm animal welfare, networked academic societies; e-collaboration; e-learning course development

INTRODUCTION

During the last years and in parallel with the increasing consumers' concern for safe and high quality food, the needs for vocational education and training on the domain of quality assurance in all stages of food production came out. Moreover, there is a need to re-educate the workforce continuously for the companies to be competitive and for persons to be attractive in the job-market and the educational model have to be changed for these learners since they will not afford for various reasons to return to the universities to have the training on Campus (Thorleif and Mikalsen, 2003). Information and communication technologies (ICTs) are playing an increasingly prominent role to the above practices by providing tools and models for the storage and reuse of digital material for teaching and learning (Friedland and Pauls, 2005). Building on a rich tradition of vocational education and training (VET) systems in Europe, the Leonardo da Vinci project entitled 'WELFOOD: Promoting quality assurance in animal welfare – environment – food quality interaction studies through upgraded e-Learning' aims to develop modern and innovative educational material to meets the current needs of vocational training of the citizen, the labour market and society on the domain of farm animal welfare, farm environment and food quality as for the interactions between them (Szücs et al., 2005). Specific topics are: ethical views on animal

welfare, assessment and improving animal welfare, impacts of the environment from and on animals, nutrient efficiency and emissions, on-farm risk assessment and HACCP, traceability and quality assurance, interactions between animal welfare, environment and food quality.

METHODOLOGY

WELFOOD *Networked Academic Society (NAS)* was defined as a group of academics with the common academic research and teaching interests on farm animal welfare collaborating towards the joined development of an e-Learning course in their field of expertise.

The members of the group were geographically dispersed (Hungary, Belgium, Poland, Greece, Estonia) and they collaborated in a face-to-face situation, but mainly by *using Information and Communication Technologies (ICTs).*

The WELFOOD NAS started the procedure with a careful analysis of the needs of the target students and agreement on a number of essential elements as: learning outcomes, content/material, delivery methods, coursework/exercises and assessment schemes. Academics having exchanged ideas and shared expertise through a networked environment enriched with ICT-tools to develop the e-course (Stamatis et al., 2006).

A set of generic quality indicators that should be applicable in both development as well as delivery of the course was also needed for the framework to be successful (Sossidou et al., 2005).

In the WELFOOD approach, the e-Learning process was evident in two phases:

- *Phase I.* First phase was concerned with the *NAS* during the development of the 'course': (1) Collecting data of academic organisations in the domain of Farm Animal Welfare, and (2) Development of database management system to organise the above data.
- *Phase II.* Second phase was concerned with both *NAS* and *Networked Student Society (NSS)* during the delivery of the course and comprised the development of information packages for potential end-users using recent findings and the results derived from the evaluation procedure (internal and external) of the quality of the course.

RESULTS AND DISCUSSION

The end result of NAS process is the WELFOOD e-Learning course uploaded to the Project Homepage (http://www.welfood.szie.hu) and upgraded in English as lingua franca and languages of the Partner countries (Estonian, Flemish, Greek, Hungarian and Polish). The course aims to train students scientifically and technically to enable them to understand and resolve problems relating to farm animal welfare, environment and food quality interactions. It focuses on farm animal welfare standards and specifications and aims to promote quality assurance in animal welfare, environment and food quality interaction studies through upgraded e-Learning. The course also aims to support new knowledge and skills in quality assurance in all phases of sustainable food production from animal origin by further improvement of curricula in vocational training. Course material consists of html texts published as web pages highlighting the important points, and downloadable power-point presentations, Word and PDF documents. A list of references and useful web links is also provided for each lesson. To obtain professional accreditation for the e-learning course, assessment is based on the student's participation in the forum and a final examination. The number of hours necessary to learn a subject area varies with the characteristics of the individual. Consideration must be given to the e-learning course in

relation to professional accreditation, which is understood to mean the recognition of an individual's level of knowledge and skills for practicing his or her profession.

The course is taught in three learning units: 1. Animal Welfare, 2. Environmental impacts on and of animals, 3. Food quality and safety. These units are the contents/tasks used to describe the project structure (Work Packages and Tasks). The learning units are delivered asynchronously through a Web server on a weekly basis. Lectures are published every week on the web server with relevant case studies and questions on the topic for students to download. Teaching Methods include tutoring and online discussions for further definition of terms and meanings and mentoring towards collaborative project work among groups of students. A collaborative environment (forum) for project work activities and discussions is provided. Students contact tutors and facilitators through e-mail for tutorials and other academic advice. A list of discussions on different topics is maintained through the bulletin board. Every month there is a synchronized session via a net meeting.

WELFOOD e-course addresses several target groups. First of all they are students who want to enroll in WELFOOD MASTER degree programs or taking single course modules. The students could be classified as: internal campus students, external students and groups of students coming from industry or public sector. The other target groups could as well be called beneficiaries. They are in addition to the students: national authorities, political decision makers and administrators, university leaders, curriculum developers and administrators and university faculty members.

Apart from being a training environment for the different target groups, the e-learning course that has been developed plays the role of a communication forum between course developers (farm animal welfare teachers, professionals and experts) and in this respect it supports the following (Sossidou et al., 2007):

- **Distributed expertise:** Access to experts, not readily available in each geographical area, is encouraged through the networked community of practice.
- **A tailor-made approach to training:** Adopting the course to special target groups is relatively easy to achieve.
- **Continuous Update:** Adopting a collaborative approach to e-learning course material is updated continuously as the needs arise.

The major benefits of NAS may (1) balance among international core curricula vs. local specialities of training organisations on animal welfare and related issues; (2) contribute to develop European dimension in the specific curricula in response to challenges; (3) promote networking for European students and teaching staff interested in the specific area; (4) facilitates the mobility among similar study programs with different training/learning approaches; (5) upgrade transparency and enhance overall quality in vocational training through international comparative evaluations, as well as assist EU cooperation foster comparability, compatibility, competitiveness and overall attractiveness of EU communication system in the disciples in question.

In addition, the outcome will support (1) the preparedness of the institution for international participation; (2) development of curricula; (3) learning through sharing experiences; (4) clarification of good practice in development of European dimension in teaching and learning.

CONCLUSIONS

By concluding, developing the WELFOOD course has outstanding benefits: (a) wider knowledge and better e-Learning training skills for each of the members of NAS and (b) higher quality course compared to similar courses delivered locally by one or only few academics. Moreover, it should be stressed that collaboration among WELFOOD partners facilitates evaluation of the course while it is being developed, as opposed to evaluation after delivery, as it usually happens.

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PLENARY LECTURES

THE ZOONOSES REGULATIONS – NEW APPROACHES TO REDUCING ZOONOTIC PATHOGENS

Klein, G.[1] and Blaha, T.[2]

[1] Institute of Food Quality and Food Safety, University of Veterinary Medicine, Bischofsholer Damm 15, 30173 Hannover, German; [2] Bakum Field Station of Epidemiology, University of Veterinary Medicine, Büscheler Str. 9, 49456 Bakum, Germany

SUMMARY

The zoonoses regulations consist of two legal acts, i.e. Regulation (EC) 2160/2003 on the control of *Salmonella* and other specified zoonotic agents, which is direct applicable in each member state, and the Directive 2003/99/EC. They are amended by specific regulations and on the food safety side by Regulation (EC) 2073/2005 on microbiological criteria for foodstuffs. This regulation defines process hygiene and food safety criteria. Targets set by the zoonoses regulation upon the data derived through baseline studies according to the zoonoses directive can result in stricter microbiological food safety criteria according to the regulation on microbiological criteria in foodstuffs.

Keywords: zoonoses, zoonotic agents, monitoring, food safety

INTRODUCTION

The so called zoonoses regulations of the European Community consists mainly of two legal acts, i.e. Regulation (EC) No 2160/2003 (EC, 2003b) on the control of *Salmonella* and other specified zoonotic agents, which is direct applicable in each member state, and the Directive 2003/99/EC (EC, 2003a) on the monitoring of zoonoses and zoonotic agents, which is a guidance act to be implemented in each member state. Meanwhile also more specific regulations have been issued with specific targets like *Salmonella* in laying hens (Regulation (EC) No 1168/2006) (EC, 2006). The goals of those legal acts are two-fold. First of all the current situation concerning important zoonotic agents within the Community should be evaluated by monitoring programs, including baseline studies. Secondly targets for prevalence rates should be set in order to achieve a reduction in the prevalence of the major zoonotic pathogens. The success of those methods will again be monitored at each step, so that targets can be set lower over time. The first goal (monitoring) is described by the Directive, the second one by the Regulation and its amendments. In the following, the approaches of the Directive and the Regulation will be described and methods and targets will be discussed.

On the food safety side the Commission Regulation (EC) No 2073/2005 (EC, 2005) on microbiological criteria in foodstuffs completes the picture. In this Regulation microbiological criteria in the process line and the endproduct are set for the main zoonotic agents and foodstuffs. Depending on the sampling site process hygiene criteria (during processing) and food safety criteria (for the endproduct) are defined. Thus, the zoonoses regulations and the Regulation on microbiological criteria in foodstuffs are linked and fill the gap between the efforts for animal

health and food safety in the primary production and the food safety issues in the food producing and marketing sector.

DIRECTIVE 2003/99/EC – THE MONITORING DIRECTIVE

The goal of the Directive 2003/99/EC on the monitoring of zoonoses and zoonotic agents is to ensure that zoonoses and zoonotic agents, their respective antibiotic resistances and foodborne outbreaks are monitored in an epidemiological way. Changing tendencies over time should be detected and possible sources be identified. The data should be sampled and analysed in a Community zoonoses report. This has been done also previously, but the analysis of the data was not systematic and there was no common approach within the member states (Hartung, 2004). Those data should then be used for a scientific microbiological risk analysis according to Codex Alimentarius (CAC, 1999).

The existing systems of the member states could be used, however if necessary, harmonisation is possible. Indeed, the responsibility to create and publish the Community zoonoses report was transferred from the EC Commission to the European Food Safety Authority (EFSA), which was established according to the basic Regulation (EC) No 178/2002 (The General Food Law) in the year 2003 (EFSA, 2007). In each member state a standardised sampling of relevant data should take place and the results should be forwarded in a standardised format to the EFSA database. EFSA will then compile a "Community Summary Report on Trends and Sources of Zoonoses, Zoonotic Agents and Antimicrobial Resistance in the European Union" for each year (EFSA, 2007). The EFSA expert panel on Biological hazards will give advice on the final report (EFSA, 2007). In Germany also a national report on the epidemiological situation of zoonoses, zoonotic agents, antimicrobial resistance and foodborne outbreaks is issued (Hartung, 2006).

There are also routine monitoring programmes implemented in member states concerning the antibiotic resistance in relation to zoonotic agents. Especially resistance patterns of zoonotic agents from animals reared for food production and from food itself are registered. Trends in the change of those resistance patterns should be detected and also the early detection of new, emerging resistances. This includes where possible also the emergence of new resistance mechanisms. In addition, differences in different geographic regions should be detectable.

The quality of these data greatly depends on the representativity of the isolates and the sample size (n). The latter factor is the denominator for the precision of the analysis.

Antibiotic resistance can be monitored successively in three areas. The monitoring can include zoonotic agents (e.g. *Salmonella* spp., *Campylobacter jejuni* and *C. coli*) from food at the retail level (incl. pork meat, beef, poultry meat, food containing raw eggs). Another source are isolates from clinically healthy animals (used for food production) concerning the same agents. In addition clinically ill animals (used for food production) can be sampled similarly.

Coordinated control programs of all member states complete the data set initiated by the monitoring Directive 2003/99/EC. So far coordinated control programs started for *Salmonella* spp. in laying hens and broiler. They will be followed by programmes dealing also with other animal species like turkey and fattening pigs and other zoonotic agents.

Those baseline studies should deliver prevalence data, which are comparable between member states and should state the real "base" line for future considerations. This baseline will serve then as the basis for future targets to be implemented by the control regulation, the Regulation (EC) No 2160/2003 on the control of *Salmonella* and other specified zoonotic agents. In the first years experience with the control programmes has to be gained. In the future those programmes should

be mandatory in order to reach the implemented target. In Germany the baseline study for *Salmonella* spp. in broiler chicken has been recently performed and published (BfR, 2006).

REGULATION (EC) NO 2160/2003 – THE CONTROL REGULATION

Regulation (EC) No 2160/2003 on the control of *Salmonella* and other specified zoonotic agents sets the scene for the establishment of Community targets, for reducing the prevalence of zoonotic agents with public health significance in animals used for food production. The targets should include as defined in the Regulation the maximum time limits within which the targets shall be reached, the definition of epidemiological units, the definition of the testing schemes necessary to verify the achievement of the targets and, where relevant the definition of the agents with public health significance. Before proposing rules on specific control methods, the Commission should consult the European Food Safety Authority (EFSA). An excerpt from the Regulation on the time limits is given in Table 1.

Table 1. Maximum time limits within which the targets for zoonotic agents should be reached according to Regulation (EC) No 2160/2003

1. Zoonosis or zoonotic agent	2. Animal population	3. Stage of food chain	4. Date by which target must be established (*)	5. Date from which testing must take place
All salmonella serotypes with public health significance	Breeding flocks of *Gallus gallus*	Primary production	12 months after the date of entry into force of this Regulation.	18 months after the date referred to in column 4
All salmonella serotypes with public health significance	Laying hens	Primary production	24 months after the date of entry into force of this Regulation.	18 months after the date referred to in column 4
All salmonella serotypes with public health significance	Broilers	Primary production	36 months after the date of entry into force of this Regulation.	18 months after the date referred to in column 4
All salmonella serotypes with public health significance	Turkeys	Primary production	48 months after the date of entry into force of this Regulation.	18 months after the date referred to in column 4
All salmonella serotypes with public health significance	Herds of slaughter pigs	Slaughter	48 months after the date of entry into force of this Regulation.	18 months after the date referred to in column 4
All salmonella serotypes with public health significance	Breeding herds of pigs	Primary production	60 months after the date of entry into force of this Regulation.	18 months after the date referred to in column 4

(*) These dates are based on the assumption that comparable data on prevalence will be available at least six months before the establishment of the target. If such data were not available, the date for the establishment of the target would be postponed accordingly.

REGULATION (EC) NO 2073/2005 – THE FOOD SAFETY REGULATION

The Regulation (EC) No 2073/2005 on microbiological criteria for foodstuffs translates the foodborne pathogen targets defined by the zoonoses control regulation (Regulation (EC) No 2160/2003) into food safety criteria. The regulation differentiates between process hygiene criteria and food safety criteria. Process hygiene criteria apply for all stages of the production process until the food leaves the process line for the retail level. Those criteria are intended to control the process hygiene. Failing of the criterion must lead to an improvement in process hygiene like intensified cleaning and disinfection. Those criteria include also indicator organisms like *E. coli*. Food safety criteria apply solely at the retail level, where the endproduct is presented to the consumer. It concerns only foodborne pathogens like *Salmonella* spp. When a criterion is not met at this stage, revocal from the market is necessary or at least heat treatment of the product, when it has not been brought to the market.

An example concerning *Salmonella* spp. in broiler for the relationship between the zoonoses directive, the zoonoses regulation and the regulation on microbiological criteria following Table 1 can be given as follows:

Within primary production should have been performed in 2006, targets should be set in 2007 following the baseline study and control actions should start in 2009. In parallel the regulation on microbiological criteria in foodstuffs is in force since the beginning of 2006 with the criterion of absence of Salmonella spp. in 10g at the retail level. With the beginning of 2010 and in parallel to the above mentioned timeframe the criterion is absence in 25g at retail level, i.e. a stricter criterion is applicable.

In conclusion targets set by the zoonoses regulation upon the data derived through baseline studies according to the zoonoses directive can result in stricter microbiological food safety criteria according to the regulation on microbiological criteria in foodstuffs.

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AVIAN INFLUENZA – CURRENT SITUATION AND FUTURE TRENDS

Terregino, C.

OIE, FAO and National Reference Laboratory for Newcastle Disease and Avian Influenza, Istituto Zooprofilattico Sperimentale delle Venezie, Viale dell'Università 10, 35020 – Legnaro, Padova, Italy

In the last 10 years many aspects of the epidemiology of avian influenza (AI) infections in poultry and other birds appear to have changed dramatically from those established in the preceding century. The number of outbreaks of the highly pathogenic disease (HPAI) has increased alarmingly in the last 10 years and, even more noticeably the impact in terms of the number of birds involved and the costs of control disease have dramatically escalated. But what has been most marked is the apparently unprecedented emergence and spread of the HPAI H5N1 virus in SE Asia and beyond which, with the zoonotic infections, has resulted in AI being considered one of the most important animal diseases, if not the most important.

Influenza viruses have segmented, negative sense, single strand RNA genomes and are placed in the family *Orthomyxoviridae*. At present the Orthomyxoviridae family consists of five genera, only viruses of the *Influenzavirus A* genus are known to infect birds.

Influenza A viruses are further divided into subtypes based on the antigenic relationships in the surface glycoproteins, haemagglutinin (HA) and neuraminidase (NA). At present 16 HA subtypes have been recognised (H1-H16) and nine NA subtypes (N1-N9). Each virus has one HA and one NA antigen, apparently in any combination. All influenza A subtypes in the majority of possible combinations have been isolated from avian species. To date only viruses of H5 and H7 subtype have been shown to cause HPAI in susceptible species, but not all H5 and H7 viruses are virulent.

For all influenza A viruses the haemagglutinin glycoprotein is produced as a precursor, HA0, which requires post translational cleavage by host proteases before it is functional and virus particles are infectious [1–3]. The HA0 precursor proteins of AI viruses of low virulence for poultry (LPAI viruses) have a single arginine at the cleavage site and another basic amino acid at position –3 or –4 from the cleavage site. These viruses are limited to cleavage by extracellular host proteases such as trypsin-like enzymes and thus restricted to replication at sites in the host where such enzymes are found, i.e. the respiratory and intestinal tracts. HPAI viruses possess multiple basic amino acids (arginine and lysine) at their HA0 cleavage sites either as a result of apparent insertion or apparent substitution [2–4–6] and appear to be cleavable by an intracellular ubiquitous protease(s), probably one or more proprotein-processing subtilisin-related endoproteases of which furin is the leading candidate [5]. HPAI viruses are able to replicate throughout the bird, damaging vital organs and tissues, which results in disease and death.

The factors that bring about mutation from LPAI to HPAI are not known. In some instances mutation seems to have taken place rapidly (at the primary site) after introduction from wild birds, in others the LPAI virus has circulated in poultry for months before mutating [7–8]. Therefore, it is impossible to predict if and when this mutation will occur. However, it can be reasonably assumed that the wider the circulation of LPAI in poultry, the higher the chance that mutation to HPAI will occur. HPAI viruses are not necessarily virulent for all species of birds and the clinical severity seen in any host appears to vary with both bird species and virus strain [9, 11]. In particular ducks rarely show clinical signs as a result of HPAI infections although there are

reports that some of the Asian H5N1 viruses have caused disease [12] and the HPAI viruses A/duck/Italy/2000 (H7N1) and A/chicken/Germany/34 (H7N1) have been reported to cause disease and death in naturally and experimentally infected waterfowl [10].

Influenza viruses have been shown to infect a great variety of birds [for reviews see 13–17], including free-living birds, captive caged birds, domestic ducks, chickens, turkeys and other domestic poultry. It was not until the mid-1970s that any systematic investigations of influenza in feral birds were undertaken. These investigations revealed enormous pools of influenza viruses to be present in the wild bird population [17, 18–20] especially in waterfowl, Family Anatidae, Order Anseriformes. In the surveys listed by Stallknecht and Shane [19] a total of 21 318 samples from all species resulted in the isolation of 2317 (10.9%) viruses. However, 14 303 of these samples were from birds of the Order Anseriformes which yielded 2173 (15.2%) isolates. The next highest isolation rates were 2.9% and 2.2% from the Passeriformes and Charadriiformes, respectively; but these compare with an overall isolation rate of 2.1% from all birds other than ducks and geese. However, studies by Sharp et al., [21], suggest that waterfowl do not act as a reservoir for all avian influenza viruses. It seems likely that part of the influenza gene pool is maintained in shorebirds and gulls, from which the predominant number of isolated influenza viruses are of a different subtype to those isolated from ducks [22].

Until the spread of Asian HPAI H5N1 (see below), HPAI viruses had been isolated rarely from free-living birds and, apart from A/tern/S.Africa/61 [23], when they had been isolated it was usually in the vicinity of outbreaks of HPAI in poultry or geographically and chronologically close to known outbreaks in poultry. The different epidemiology of the Asian H5N1 HPAI has led to several groups re-examining the understanding of AI virus transmission. In particular the change in the primary route of transmission from faecal/oral to the respiratory route in land birds, especially minor poultry species such as quail and pheasants has been considered significant in the epidemiology of that virus, especially in its spread to mammals [24–26]

The emergence of HPAI H5N1 virus in SE Asia and its spread across Asia and into Europe and Africa is unprecedented in the virological era. The apparent progenitor virus for the subsequent outbreaks of HPNAI of H5N1 subtype was obtained from an infection of commercial geese in Guandong province PR China in 1996 [27]. In some reports it has been considered that the virus continued to circulate in southern China primarily in domestic ducks and showing some genetic variation [28]. This apparent low-level, but probably endemic, situation changed dramatically in December 2003 to February 2004 when suddenly eight countries in E and SE Asia reported outbreaks of HPNAI due to H5N1 virus [28]. Although there seemed to be some success in controlling the outbreaks in some countries, it appeared to re-emerge in a second wave in July 2004 onwards. Malaysia reported an outbreak in poultry in August 2004 and became the ninth country in the region to be affected [29]. The virus appeared to affect all sectors of the poultry populations in most of these countries, but its presence in free range commercial ducks, village poultry, live bird markets and fighting cocks seemed especially significant in the spread of the virus [28, 27, 30].

If HPAI virus becomes widespread in poultry, especially in domestic ducks that are reared on free range, spill-over into wild bird populations is inevitable. In the past such infections have been restricted to wild birds found dead in the vicinity of infected poultry, but there has always been concern that infections of wild birds in which HPAI virus caused minimal or no clinical signs (i.e. ducks) could result in spread of the virus over large areas and long distances. Outbreaks affecting many wild bird species at two waterfowl parks in Hong Kong were recorded in 2002 [31] and further, possibly more significant, outbreaks in wild migratory birds were reported in China and Mongolia in 2005. In particular it was suggested that presence of virus in migratory birds at Lake

Qinghai in Western China could be the means by which the H5N1 [32, 33] virus could spread West and South.

There is no certain evidence that wild birds were responsible for the introduction into Russia but HPAI H5N1 virus, genetically closely related to isolates obtained at Lake Qinghai, reached poultry there in the summer of 2005. Whether spread from there to other Western Asian and some Eastern European countries occurred or virus was introduced independently is not clear, nor is whether spread was associated with movements of poultry or wild birds, probably both were involved, but during 2005 to the beginning of 2006 genetically closely related H5N1 viruses appeared in a number of countries in the region.

Reports of HPAI H5N1 virus infections continued in Europe and in Africa during the first six months of 2006 and by the end of 2006 56 countries in Asia, Europe and Africa had reported HPAI caused by H5N1 virus to the World Organisation for Animal Health (OIE) since the end of 2003 [29].

The epidemiology of AI has changed in the last 10 years, not only because of the failure to control and eradicate infections in poultry due to HPAI H5N1 viruses, but also because the continued development and industrialisation of the poultry industries throughout the World has meant that AI infections, especially HPAI outbreaks have had a far greater impact in terms of spread and loss of birds than in earlier years. In addition, in the past the spread of HPAI virus to wild birds has not been recorded on the scale reached by the Asian HPAI H5N1virus. Whether the virus is likely to become or remain endemic in some species of wild birds or would gradually die out if there was no further spread from infected poultry is not clear.

This change in the ecology and epidemiology of AI infections requires the urgent generation of new knowledge on issues related to epidemiology, pathogenesis and control. The Asian HPAI H5N1 viruses have spread to three continents, with completely different agricultural, ecological social and economic backgrounds. This in turn is likely to result in the establishment of different mechanisms by which the virus may be perpetuated in a given area. The generation of such cycles will be influenced by the diversity and availability of hosts in that area. As the virus encounters new hosts – within and outside the Class *Aves*, it may well acquire mutations that may reflect replication advantages in one or more species, but affect the pathogenicity and transmissibility in those and other species.

In view of the zoonotic potential it would appear important that the Asian HPAI H5N1 virus is eliminated from poultry at least and not just contained by the use of vaccination, as has been the strategy with other poultry viruses, especially Newcastle disease virus [34], which remains endemic in many parts of the world. Additionally, the application of control programmes encompassing vaccination may result in the generation of strains that have progressively drifted away from the original antigenic profile [35]. To date it is unclear how the immunological pressure generated by the variety of seed strains contained in available and planned veterinary vaccines will affect the antigenic properties of isolates.

The results of these two driving forces in the genetic and antigenic profile require careful monitoring of viral strains and a close collaboration between the parties involved in the crisis management. The monitoring effort should aim at the collection and characterisation of strains in order to identify genetic mutations and antigenic properties. Information should be collated and made available to the international scientific community, so that those involved in both animal and human health are fully informed of the current situation.

Efforts to bring about control and eradication internationally will have to take into account the extremely complex situation especially in any given geographical location the characteristics of the poultry producing sector in its entirety, the eco-epidemiological situation, the response

capacity of the veterinary infrastructure and the availability of adequate resources. These features must be integrated with the social environment, including those linked to the rearing of birds for recreational and farming purposes. It is possible that in some areas control and eradication will never be achieved and great changes in the way poultry are reared and they and their products marketed will be necessary.

For this reason, international organisations that govern trade regulations and animal disease control should establish a set of guidelines so that control programmes may be "accredited" and consequently internationally recognized. Such a policy would appear to have several practical advantages, ultimately resulting in an improved crisis management. These include rapid approval of established control programmes, constant update on the field situation, feedback of information on successes and failures, harmonisation of protocols and systems and public availability of control and eradication programmes. In this way, even inexperienced countries can maximise the outcome of other experiences to combat this infection in an educated manner – thus avoiding wastage of resources and time.

At least two AI subtypes, H5N1 and H9N2, both of which have zoonotic implications are currently endemic in vast areas of the world. It is impossible to predict whether either of them will represent the progenitor of the next human pandemic virus. Certainly, both of them are causing losses to the poultry industry and H5N1 is also causing the loss of human lives and the reduction of the livelihood of rural establishments. The extensive and uncontrolled circulation of these strains could result in catastrophic consequences for both human and animal health and therefore requires an extraordinary and coordinated international effort so that control and eradication can be successfully managed and achieved.

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SIGNIFICANCE OF FEED-BORNE *FUSARIUM* MYCOTOXINS ON LIVESTOCK HEALTH AND REPRODUCTION

Smith, T.K., Diaz-Llano, G., Korosteleva, S.N. and Yegani, M.

Department of Animal and Poultry Science, University of Guelph, Guelph, Ontario, Canada, N1G 2W1.

SUMMARY

Reproducing pigs, broiler chickens and dairy cows are all adversely affected by feed-borne *Fusarium* mycotoxins. The most significant economic effect is likely immunosuppression resulting in increased frequency of secondary mycotoxic diseases, lack of response to medications and failure of vaccination programs. Contaminated feedstuffs should be fed only with caution.

Keywords: *Fusarium* mycotoxins, reproduction, pigs, broiler breeders, dairy cows, immuno-suppression

INTRODUCTION

Mycotoxins are fungal metabolites which can reduce performance and alter metabolism of livestock and poultry (Wannemacher *et al*., 1991). The pathological states arising from the consumption of feeds contaminated with mycotoxins are mycotoxicoses. Mycotoxins can be formed in the field preharvest and may continue to be formed under suboptimal storage conditions postharvest. High moisture content often predisposes feedstuffs to fungal growth and mycotoxin formation. Temperature is another key factor. Some fungi, such as *Aspergillus flavus*, are usually found in tropical and semi-tropical climates. This mould produces the carcinogenic hepatotoxin aflatoxin. *Fusarium* fungi, however, are more common in temperate climates and *Fusarium* mycotoxins are likely the most common mycotoxins on a global basis (Wood, 1992).

There are many reports of the effects of *Fusarium* mycotoxins on growth rates and metabolism in livestock and poultry but less research has been devoted to the effects of *Fusarium* mycotoxins on reproduction. This is no doubt a reflection of the complexity of such experiments. Ruminant animals have been considered to be more resistant to the effects of feed-borne mycotoxins because of the detoxifying potential of rumen microorganisms. Charmley *et al.* (1993), Ingalls (1996) Trenholm *et al*. (1985) reported few adverse effects of deoxynivalenol (DON, vomitoxin) contaminated feed on performance of lactating and non-lactating dairy cows. Friend *et al*. (1983) fed 3.45 mg DON / kg feed to gestating sows and noted a significant reduction in feed intake, body weight gain, fetal length and fetal weight at 20–54 days of gestation. Chavez (1984) fed sows naturally-contaminated wheat to provide up to 3.29 mg DON / kg feed for the last 90 days of gestation. No effect was seen on litter size or piglet weight at birth but reduced feed intake and weight gain were observed. There have been even fewer reports of the effect of feed-borne *Fusarium* mycotoxins on reproduction in broiler breeder chickens. Brake *et al*. (1999) fed up to 20 mg diacetoxyscirpenol (DAS)/kg feed to breeder hens and roosters and observed decreased fertility in broiler breeder males.

Experiments were conducted, therefore, to determine the effect of feed-borne *Fusarium* mycotoxins on reproduction and metabolism in sows, broiler breeder hens and roosters and lactating dairy cows.

MATERIALS AND METHODS

FEEDING TRIAL WITH DAIRY CATTLE

A study was conducted to determine the effect of feeding lactating dairy cows TMR containing wheat, corn and hay naturally-contaminated with *Fusarium* mycotoxins. A total of 18 mid-lactation Holstein cows (6 cows per diet) with an average milk production of 30 – 35 kg / day were fed for 56 days. Treatments included: (1) control (2) contaminated grains (3) contaminated grains + 0.2% GMA (Mycosorb, Alltech Inc., Nicholasville, KY), a polymeric glucomannan mycotoxin adsorbent. DON was the major contaminant and was found at up to 3.6 mg/kg in TMR dry matter. Zearalenone and 15-acetyl DON were found in lesser concentrations. Body weight, body condition score, milk production, milk composition, somatic cell count (SCC), blood serum chemistry, haematology, total immunoglobulin (Ig) count and coagulation profile were measured.

FEEDING TRIALS WITH SWINE

A study was conducted to determine the effects of feeding a blend of corn and wheat naturally-contaminated with *Fusarium* mycotoxins to gestating and lactating sows. A total of 36 first parity Yorkshire gilts (12 per treatment) were housed in individual stalls for 21 days before farrowing and 21 days after farrowing. During gestation, feed was restricted to 2.4 kg/pig/day. Treatments included (1) control (2) contaminated grains (3) contaminated grains + 0.2% GMA. DON was the major contaminant and was present at 5.7 mg/kg in contaminated diets and 0.2 mg/kg in the control diet. Zearalenone and 15-acetyl DON were found in lesser concentrations. Parameters measured included body weight change, feed consumption, numbers and weights of piglets born, numbers of stillborn and mummified piglets, milk composition, and viability of piglets until weaning, blood chemistry and weaning to oestrus interval.

FEEDING TRIAL WITH POULTRY

A study was conducted to determine the effects of feeding a blend of corn and wheat naturally-contaminated with *Fusarium* mycotoxins to broiler breeder hens and roosters. Forty-two 26-wk-old broiler breeder hens and nine roosters (Ross 308) were weighed and randomly assigned to individual wire cages serving as 14 and 3 replicates respectively for each of the three treatment groups. Feed consumption of hens was restricted to 133 g/bird/d increasing to 155 g/bird/d at the end of the experiment. Diets included (1) control (2) contaminated grains (3) contaminated grains + 0.2% GMA. The major contaminant was DON which was found at about 13 mg/kg in contaminated diets and 0.2 mg/kg in the control diet. Zearalenone and 15-acetyl DON were again found in lesser concentrations. Contaminated rooster diets contained an average of 7.8 mg/kg DON with the control diet containing 0.9 mg/kg. Hens were individually inseminated three times during the week before egg collection with 50 ul of fresh pooled semen from roosters fed corresponding diets. Experimental parameters measured included feed consumption, body weight change, egg production, egg weight, shell deformity, albumin height, yolk weight, shell weight, shell thickness, weights of liver, spleen and kidney, biochemistry, haematology, serology, hatchability, progeny performance and rooster fertility.

DETERMINATION OF DIETARY MYCOTOXIN CONCENTRATIONS

Dietary contents of 19 mycotoxins including DON, 3-acetyl DON, 15-acetyl DON, nivalenol, T-2 toxin, iso T-2 toxin, acetyl T-2 toxin, HT-2 toxin, T-2 triol, T-2 tetraol, fusarenone-X, diacetoxyscirpenol (DAS), scirpentriol, 15-acetoxyscirpentriol, neosolaniol, zearalenone, zearalenol, aflatoxin and fumonisin were analyzed by gas chromatography and mass spectrometry (Raymond *et al*., 2003). The detection limits were 0.2 mg/kg with exception of aflatoxin and fumonisin which were detected at 0.02 and 2 mg/kg respectively.

RESULTS AND DISCUSSION

FEEDING TRIAL WITH DAIRY CATTLE

There was no effect of diet on feed consumption, body weight change, body condition score, milk production, milk composition or milk somatic cell count. Total serum protein and globulin concentrations were increased after 42 days of feeding in cows fed contaminated TMR while albumin:globulin ratio decreased compared to controls (Table 1). Cows fed contaminated TMR + GMA were not significantly different from controls. These changes might reflect the beginning of liver damage due to mycotoxin exposure. The changes in serum proteins do not appear to be a sign of acute inflammation as there was no elevation in other markers of inflammation.

The feeding of contaminated TMR resulted in a continuous elevation in serum urea concentrations throughout the experiment and this effect was prevented by dietary supplementation with GMA. It is not clear whether the elevated blood urea concentrations are due to the effect of DON and other trichothecene mycotoxins in inhibiting protein synthesis in rumen microbes or in inhibiting hepatic protein synthesis.

The feeding of contaminated TMR also significantly reduced serum IgA concentrations after 36 days of feeding and this was prevented by dietary supplementation with GMA. This likely reflects the immunosuppressive effects of *Fusarium* mycotoxins as has been described in monogastric species.

It was concluded that feed naturally contaminated with *Fusarium* mycotoxins, even in low concentrations, can affect metabolic parameters and immunity of dairy cows and the feeding of GMA can prevent many of these effects

FEEDING TRIALS WITH SWINE

There was no effect of diet on average daily feed intake of gilts in gestation (Table 2). Weight gain and gain:feed ratios, however, were reduced by the feeding of contaminated grains and this was prevented by the feeding of GMA. Serum chemistry was unaffected by diet. The percentage of stillbirths was higher and the total piglets born was lower for gilts fed contaminated grains compared to those fed contaminated grains + GMA. There was no effect of diet on frequency of mummies at birth, total piglets born or body weight of piglets at birth. In the lactation period, feed intake and weight gain were reduced by diets containing contaminated grains (Table 3). Blood chemistry, milk composition and piglet weights at weaning were not affected by diet. There was a strong trend, however, to increase weaning to oestrus interval when sows were fed contaminated grains.

It was concluded that the feeding of grains naturally contaminated with *Fusarium* mycotoxins to gestating and lactating sows results in increased numbers of stillborn piglets but piglets that are born alive are viable and thrive throughout the lactation period. This is achieved, however, by a

marked depletion of body reserves resulting in trend towards increased weaning to oestrus intervals.

FEEDING TRIALS WITH POULTRY

There was no effect of diet on feed consumption or feed efficiency (feed consumed / egg produced) and body weights were also not affected (Table 4). There was a trend towards reduced egg production in birds fed the contaminated grains and this was significant in week 6. The feeding of contaminated grains did, however, reduce eggshell thickness after 4 weeks and this was accompanied by an increase in early (1–7 d) embryonic mortality. These effects were prevented by the feeding of GMA. It has been demonstrated that shell thickness affects moisture loss during incubation prompting early embryonic mortality. There was no effect of diet on other egg parameters including weight, yolk weight, albumen height, eggshell deformity or eggshell weight. Weights of liver, spleen and kidney were also not affected by diet. There was no effect of diet on weight or viability of newly hatched chicks.

The feeding of contaminated grains decreased serum antibody titres against infectious bronchitis virus after 12 weeks and this was prevented by the feeding of GMA. There was no effect of diet, however, on serum antibody titres against Newcastle disease virus. The absence of the effect of diet on titres against Newcastle disease virus is likely due to the fact that Newcastle disease is not endemic in Canada. Reduced antibody titres against infectious bronchitis is a reflection of the immunosuppressive properties of the trichothecene mycotoxins.

Rooster semen volume and sperm concentration, viability, motility and relative weights of testes were not significantly affected by diet.

CONCLUSIONS

It can be concluded that there are adverse effects of feed-borne *Fusarium* mycotoxins on reproduction in swine, poultry and dairy cows with the severity declining in that order. These effects can largely be prevented by the feeding of GMA. This has important economic consequences when wide spread contamination of the feed supply forces the feeding of contaminated grains or when favourable pricing prompts the intentional feeding of contaminated materials.

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Table 1. Effect of feeding TMR naturally-contaminated with *Fusarium* mycotoxins on production and metabolism of dairy cows

Diet	Feed Intake (kg/cow/day)	Milk Production (kg/cow/day)	SSC (sc/ml x 10^3)	Serum IgA (g /L serum)	Serum Urea (mmol/L)
Control	48.5	30.0	64.56	0.35	5.3
Contaminated	49.5	34.0	57.25	0.16	6.3
Contaminated + 0.2% GMA	44.4	28.9	40.88	0.27	5.5
Control vs	NS[1]	NS	NS	0.01	0.01
Contaminated					
Control vs	NS	NS	NS	NS	NS
Contaminated + GMA					

[1]Not significant (P>0.05)

Table 2. Effect of feeding blends of grains naturally-contaminated with *Fusarium* mycotoxins on performance of gestating gilts[1]

Diet	ADFI (kg/d)	ADG (kg/d)	G:F %	Stillbirths %	Born Alive
Control	2.41^{a}	1.14^{a}	0.37^{a}	$6.27^{a,b}$	$90.5^{a,b}$
Contaminated grains	2.12^{a}	0.62^{b}	0.19^{b}	15.52^{b}	80.8^{b}
Contaminated grains + 0.2% GMA	2.15^{a}	$0.80^{a,b}$	$0.37^{a,b}$	4.6^{a}	95.4^{a}

[1]From Diaz-Llano and Smith, 2006.
[a,b,c]Means within a row with different superscripts are different (P<0.05).

Table 3. Effect of feeding blends of grains naturally-contaminated with *Fusarium* mycotoxins on performance of lactating sows[1]

Diet	ADFI (kg/d)	ADG (kg/d)	Weaning To Estrus Interval (d)
Control	4.87[a]	0.050[a]	6.33[a]
Contaminated grains	3.56[b]	–0.592[b]	15.00[a]
Contaminated grains	3.43[b]	–0.465[b]	15.33[a]

[1] From Diaz-Llano and Smith, 2007
[a,b,c] Means within a row with different superscripts are different (P<0.05)

Table 4. Effect of feeding blends of grains naturally-contaminated with *Fusarium* mycotoxins on performance of broiler breeder hens[1]

Diet	Egg Production (%)	Eggshell Thickness (um)	Early Embryonic Mortality (%)	IBV titre
Control	84.3	32.1	5.4	12,653
Contaminated grains	78.8	30.1	21.5	8,012
Contaminated grains + 0.2% GMA	86.7	31.5	2.3	9,340
Control vs	NS[2]	0.04	0.03	0.02
Contaminated				
Control vs Contaminated + GMA	NS	NS	NS	NS

[1] From Yegani et al., 2006
[2] Not significant (P>0.05)

ASSESSMENT OF ENVIRONMENTAL EFFECTS OF AIRBORNE EMISSIONS AND WASTE EFFLUENTS FROM LIVESTOCK PRODUCTION

Hartung, J.

Institute for Animal Hygiene, Animal Welfare and Behaviour of Farm Animals, University of Veterinary Medicine Hanover, Germany

SUMMARY

Modern animal production is increasingly regarded as a source of solid, liquid and gaseous emissions which can be both a nuisance and environmentally harmful. Solid and liquid manure contain nitrogen and phosphorus, possibly heavy metals (e.g. zinc, copper) and drug residues which are passed to the environment during grazing or spreading of manure. Aerial pollutants such as odours, gases, dust, micro-organisms and endotoxins (bioaerosols) can compromise the respiratory health of farmers, animals and nearby residents, contribute to soil acidification (ammonia) and global warming (methane) and can travel several 100 m between livestock buildings with the risk of transmitting infectious agents.

Keywords: livestock production, environmental impact, bioaerosols, waste effluents, zinc, copper, antibiotics

INTRODUCTION

Modern animal production is increasingly regarded as a source of solid, liquid and gaseous emissions which can be both a nuisance and environmentally harmful. Solid and liquid manure and waste water contain nitrogen and phophorus which are the most important plant nutrients, but are harmful when applied to agricultural land in excess amounts thereby leading to pollution of ground water by nitrates, surface water with phosphorous (causing eutrophication) and soil with heavy metals such as zinc and copper which are used as growth promoters in the feed stuff. A third group of potentially hazardous effluents are drug residues, such as antibiotics, which may be present in the excreta of farm animals after medical treatment and which are passed to the environment during grazing or spreading of animal manure where they may conceivably contribute to the formation of antibiotic resistance in certain strains of bacteria. The same risk arises when sludge and waste water from sewage plants containing residues of antibiotics and other drugs from human consumption are discharged as fertiliser in the soil and water body.

The most important aerial pollutants are odours, gases, dust, micro-organisms and endotoxins, also called bioaerosols, which are emitted by way of the exhaust air into the environment from buildings and during manure storage, handling and disposal as well as grazing. More than 130 different gaseous compounds have been identified in the air of animal houses, which are a major source of these pollutants.

This paper gives a brief survey on the most important effluents from livestock farming and tries to assess its impact on the environment.

IMPACT OF AIRBORNE EMISSIONS

Aerial pollutants can give cause for concern for several reasons:

Firstly, there is strong epidemiological evidence that the health of farmers working in animal houses may be harmed by regular occupational exposure to air pollutants.

Secondly, an animal's respiratory health may be compromised by these pollutants. In some herds, half of all slaughter pigs may show signs of pneumonia, pleuritis or other respiratory disease. In broilers, about 30% of the birds which are rejected at meat inspection show lung lesions.

The third reason for concern is that aerial pollutants from livestock contribute to soil acidification (ammonia, NH_3) and global warming (e.g. methane, CH_4, nitrous oxide, N_2O). For example, animal production emits about 750,000 t of NH_3 per year in Germany. About 20% of global methane production originates from ruminants. Relative to carbon dioxide (CO_2), the amounts of CH_4 and N_2O in the atmosphere are low, but their global warming potential (GWP) is 21 and 310 times higher than that of CO_2, respectively. The total global emissions are estimated at 535 Tg (CH_4) and 17.7 Tg (N_2O) per year. About 45% of the methane production originates from agriculture, and nearly 20% comes from animal production. N_2O emission from anthropogenic sources is around 8 Tg per year and 6.2 Tg from livestock production. There are substantial uncertainties in all of these estimates because there are large variations in emission rates mainly due to the many influencing factors such as temperature or substrate or keeping conditions. While emission amounts of CH_4 from ruminants are relatively well known there is a considerable lack of knowledge for other species. Similarly, the reliability of the N_2O data is still poor. Animal production systems which use straw seem to release distinct higher amounts of N_2O than those employing liquid manure systems. This may result in conflicts with welfare policies introducing animal friendly and littered keeping systems. It seems necessary to enhance more detailed research in sources and sinks of these gases and that the national and international emission inventories are regularly up-dated in the light of new findings.

Fourthly, particulate emissions, such as dust and microorganisms, from livestock buildings may be a source of complaint from people living in the vicinity of livestock farms. The travel distance of viable bacteria from animal houses via the air is presently found several 100 m downwind of animal buildings; *Mycoplasma* species may travel at least 400 m. From epidemiological modelling it is known that the virus causing Mouth and Foot Disease can be transmitted over more than 75 km while in an airborne state. In a recent field study about 4000 cfu/m³ of staphylococcae were found nearly 500 m downwind a broiler barn (Schulz 2007) as shown in Figure 1. There is a need for dispersion models for particulate pollutants in animal farming.

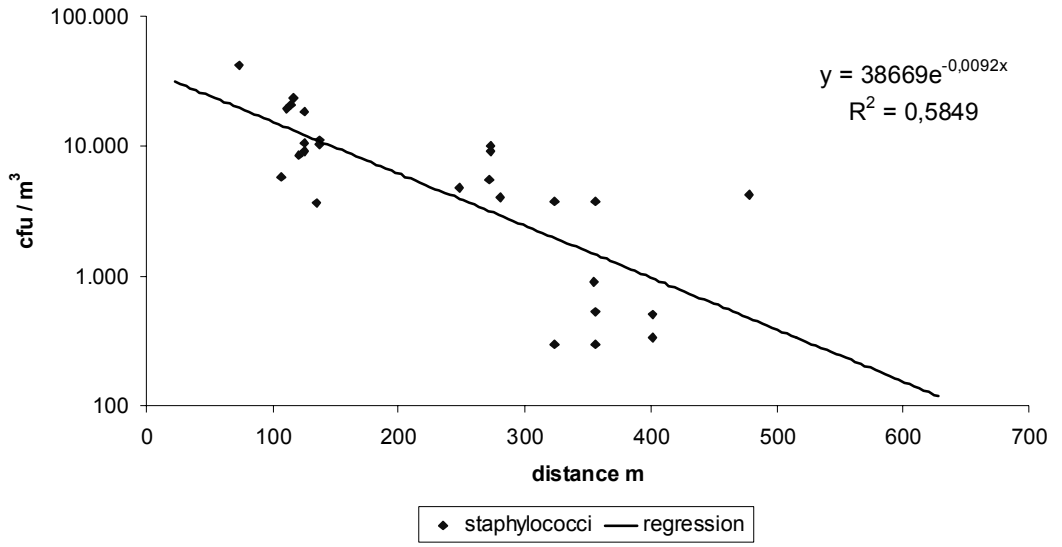

Figure 1. Decreasing concentration of airborne staphylococci in prevailing wind direction with increasing distance to the emitting broiler barn with forced ventilation at outdoor wind velocities between 1,7 and 6,3 m/s. n=26

ANTIBIOTIC RESIDUES

Little is known about the occurrence, the fate and possible effects of drugs in the environment (Kümmerer 2001, Daughton 1999). With a special focus on drugs used in human medicine, it has been established that these compounds mainly reach our surface waters via the effluents of sewage treatment plants. Today, up to 80 compounds have been identified and quantified in the low ppt to ppb ranges (Heberer 2002). Studies performed in the UK, Denmark, Germany, and the US revealed, that these agents represent a new class of organic environmental contaminants worldwide. Effects discussed by the entry of these compounds into the aquatic environment may be the spread of antibiotic resistance or effects on the endocrine system because of the hormonally nature of some of these compounds (Daughton 1999).

Only few routes have been identified so far for the entry of veterinary drugs into the environment. Recently tetracyclines in farmland in concentrations up to 300 µg/kg were found using sophisticated analytical LC-MS-MS techniques. It demonstrates that these antibiotics are persistent and can accumulate in soil after repeated fertilizations with liquid manure. Leaching of these compounds into deeper soil segments or into groundwater depends on the sorption capacity of the drugs in the first 30–40 cm of soil (Hamscher 2000 and 2002). Only limited information exists also on effects of these drugs onto the soil microorganisms. Because the soil microorganism community is a very complex system with at least 95% of unknown bacteria living in this compartment, investigations on this field are hardly to perform.

Tetracyclines and several other veterinary drugs (e.g. various sulfonamides, tylosin) are used in huge amounts not only within the EU (Anonnymus 2001) but also in the US (Kolpin 2002) and in China, Southeast Asia and Russia. They are or have been used for many years as feed additives, for prophylactic, metaphylactic, and for therapeutic purposes. Usually, the drugs are applied via

the animal feed. In intense pig production all these drugs are used and especially this production system has been known for many years as an emitting source for dust (Hartung 1997 and 1998) with the result, that this high dust exposure in animal confinement buildings has been considered as a respiratory health hazard (Nowack 1998, Iversen 2000). Recent investigations show that dust in piggeries can contain various antibiotics including tetracyclines, sulfonamides, tylosin and chloramphenicol (Hamscher et al. 2003). This indicates a new entrance route for veterinary drugs into the environment. Adverse effects on animal and human health resulting from the exposure to dust highly contaminated with antibiotics cannot be excluded and should be taken into consideration in future research.

IMPACT OF SOLID AND LIQUID EFFLUENTS

The substances which are detrimental to soil and water are those found in animal excrement containing nitrogen (N) and phosphorus (P, as phosphates). In addition there can be residues in feed, such as zinc, copper or antibiotics. Nitrogen and phosphates are important plant nutrients, which if applied properly can be used in commercial fertilizers with no adverse effects on the environment. These substances constitute a danger only if they are applied in too high amounts and at times outside the growing season. Nitrate can migrate through the soil in the groundwater. Nitrate-enriched drinking water can induce cyanosis, particularly in infants, and is suspected of having a role in stomach cancer. Nitrates and phosphates can also accumulate in surface water due to over-fertilization with liquid or solid manure or via rainwater runoff from freshly fertilized fields, and cause eutrophication of natural bodies of water. The feces of one pig corresponds to ca. three human-equivalent-units (HEU), which means that a body of water contaminated with these substances must have the regenerative power to process the equivalent of ca. 350 liters of sewage from humans (which produce ca. 120 l waste water/person/day).

The use of zinc (Zn) and copper (Cu) in piglet feed has increased recently, as they serve as trace elements necessary for a number of biological functions. At higher dosages they also have pharmacological effects and can prevent diarrhea and generally increase productivity. At present the annual transfer of zinc and copper from pig slurry onto agricultural areas comprises ca. 0.8 kg Zn and 0.4 kg Cu per hectare. This is four times the amount extracted in crop harvest for zinc and up to 20 times that for copper. It is vital that the input of these heavy metals does not exceed the amount extracted, as they will otherwise accumulate in soil and plants. Excessive intake of Zn and Cu can lead to serious poisoning in animals.

Under the existing legal fertilizer regulation, in the application of waste from animal production and of sewage sludge the nitrogen, phosphorus, zinc and copper input must not be greater than the amount withdrawn. Such balanced fertilization has been practiced with more and more success in recent years, as application of fertilizers must be preceded by analysis of the soil and of the substances to be applied.

SYNOPSIS OF ENVIRONMENTAL EFFECTS OF POLLUTANTS FROM LIVESTOCK SOURCES

Table 1 summarizes our present knowledge of the impact of emissions from livestock farming on farm livestock and man and the distance over which the emissions may have effects. Besides effects on animal and man local, regional and global impacts are characterised. Odours are

relevant closer to animal houses only. Ammonia can act directly on needles and leaves of trees close to sources where high amounts are released. It also causes damage in the far environment by over fertilizing soils and water and contributes to the decay of forests (via acid rain). Indoors, ammonia is an irritant for the respiratory tract of man and animal. Hydrogen sulphide is noticed as a prominent odorous compound outside animal houses. Occasionally indoors it can be fatal to animals and man at very high concentrations after the release of high amounts, e.g. when old liquid manure is agitated. Methane and nitrous oxide contribute to the greenhouse effect, but do not cause significant problems indoors. Little is known about the fate of dust, microorganisms and endotoxins outside livestock buildings, although there is some concern that these compounds may cause a nuisance to the population living in the vicinity of animal enterprises, particularly in areas with high animal densities. Nitrate and its product nitrite can cause pollution of ground and drinking water. The effects are local and the impact on human health is low. Together with phosphate both nutrients can enhance eutrophication of surface waters. Zinc and copper, which are increasingly used as growth promoters instead of antibiotics in animal feed, are accumulating e.g. in pig liver and locally in soils and plants that then cause health problems in grazing sheep. Not much is known about the fate of veterinary drugs such as antibiotics in the environment which are excreted with the faeces. There is some concern that they may contribute to the development of drug resistance in bacteria.

Table 1. Environmental impact from livestock sources

Compound	Impact on People	Impact on livestock	Impact on ecosystems	Local	Regional	Global
Odour	nuisance	no	no	yes	(yes)	no
Ammonia NH_3 (irritant)	indoors high	indoors high	high	high	yes	low
Hydrogen sulphide H_2S	toxic indoors	toxic indoors	no	odour	?	?
Methane CH_4	no	no	global	no	(no)	yes
Nitrous oxide N_2O	no	no	global	low	low	yes
Dust/PM10	resp. health allergy		low	yes	yes	?
Bacteria/Virus	infections	infections	no	yes	yes?	no
Endotoxin	yes	yes	no	yes	(yes)	no
Nitrate in drink. water	yes low	eutroph.	yes	yes	yes	
Phosphate	no	no	eutroph.	yes	yes	yes
Copper/Zinc	low (pig liver)	yes (sheep)	yes (soil)	yes	yes	yes
Vet drugs	resistance?	resistance?	?	?	?	?

CONCLUSIONS

- Livestock farming causes significant emissions such as nitrate, phosphate, heavy metals and also antibiotics in manure and liquid effluents as well as odour, gases, dusts, microoganisms and endotoxins in the exhaust air from animal houses, from manure storage facilities, during application of manure and during grazing.
- These effluents can have distinct impacts on air, water, soil, biodiversity in plants, forest decay and also on animal and man.
- There are indoor health effects on man and livestock (ammonia, hydrogen sulphide, bioaerosols) and impacts on the local, regional and global environment.

- Odour, bioaerosols, ammonia, nitrogen, phosphorous and heavy metals may either have a local or a regional impact. Gases such as methane and nitrous oxide contribute to global warming.
- There is equally a lack of knowledge on the airborne transmission of infectious agents such as virus and microorganisms between farms.
- Little is known on the role of drugs such as antibiotics in the environment. There is concern that these residues may contribute to the development of bacterial resistance.
- Local and regional environmental problems are enhanced by high animal densities, insufficient distances between farms and to residential areas.

RECOMMENDATIONS

1. Adequate and efficient feeding regimes are required with minimal wastage of nitrogen and phosphorous and limited use of growth promoters.
2. The development of low emission production systems should be encouraged including mitigation techniques, e.g. biofilters, bioscrubbers, covered manure pits and shallow manure application.
3. The administration of drugs has to be restricted to the treatment of diseases only. The fate of the drugs in the environment has to be investigated.
4. There is an urgent need to establish safe distances between farms and to residential areas to prevent transmission of harmful substances. This should become an essential part of local and regional planning.
5. Environmental standards for animal production should be established and applied to all European countries.
6. A systematic environmental risk analysis is required to compare different production systems and different regions worldwide.
7. For the realization of these aims the cooperation of farmers, agricultural engineers, veterinarians and governmental agencies is necessary.

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ANIMAL WELFARE AT TRANSPORT AND AT SLAUGHTER OF LIVESTOCK AND POUTRY

Manteca i Vilanova, F.X.

School of Veterinary Science, Universitat Autònoma de Barcelona, 08193 Bellaterra (Barcelona) Spain; xavier.manteca@uab.es

Scientific interest in animal welfare has rapidly grown in recent years. This has been largely due to the fact that consumers demand that animals are reared, transported and slaughtered in a humane way (Appleby and Hughes, 1997). If progress is to be made in this area, animal welfare has to be defined in a way that it can be scientifically assessed. Animal welfare can only be properly assessed if several measured are taken into account. The objective of this talk is to illustrate some of the main issues related to the welfare of animals at transport and slaughter by reviewing the main measures that can be used to assess welfare and the variables that have been shown to affect them.

Transport and slaughter are critical from an animal welfare standpoint. During transportation animals are exposed simultaneously to a variety of stressors in a relatively short period of time (Grandin, 1993). Such stressors include fasting and water deprivation, mixing of unacquainted individuals, handling by humans, exposure to a novel environment, noise and vibration, forced physical exercise and extremes of temperature and humidity (Sainsbury and Sainsbury 1988). All these factors contribute to activate the stress response through different physiological pathways. The stress response is known to be additive, i.e., the higher the number of simultaneous stress factors, the bigger the response.

Some of the main parameters used to assess welfare during transport are mortality, injuries, plasma levels of glucocorticoids, heart rate, acute phase proteins and behavioural changes. Mortality is a clear measure of poor welfare, not only because animals that die have obviously failed to cope, but also because high losses in a given environment show that even those individuals that do not die may have serious difficulties to cope. Mortality has been often used as an indicator of welfare problems during transport and studies looking at mortality have provided useful information. For example, it has been shown in pigs that halothane gene frequencies have a major effect on mortality during transport and lairage. In one study, out of 107 pigs that died during transport or lairage, 71% were nn and 24.3% were Nn. The frequency of death within the NN, Nn and nn genotypes was 0,02%, 0,09% and 2,29% respectively. Thus, according to this study, removing Nn and nn pigs from the population would result in an eleven-fold reduction in mortality rate during transport and lairage (Fàbrega et al., 2002).

Other studies have looked at the effect of journey duration and conditions on mortality rates. In broilers, for example, longer journeys to processing plants seem to be associated with higher mortality and in one study it was found that mortality was 80% higher in journeys exceeding 4 hours in duration than in those shorter than 4 hours (Warriss et al., 1992).

Injuries also provide information about the welfare of the animals during handling, transport and lairage. Skin damage on the carcass is assessed by visual inspection at the slaughter line. There are several scales that can be used to carry out such an assessment (Barton-Gade *et al*., 1996). Assessment of skin lesions at the slaughter line not only helps to determine number of marks on the carcass, but also may recognize the source (fighting, rough handling, overcrowding or poor facilities design) according to the anatomical location and damage type. Old wounds may

be recognised as scars, and may be indicative of some animal welfare problem on the farm. Fresh wounds may indicate damage due to fighting during transport and lairage.

Broken bones in laying hens are amongst the most painful injuries during transport and are therefore an important welfare concern (SCAHAW, 2002). In all species, bruising, lacerations and blemishes can be scored on the carcasses and used to assess welfare during transport and lairage (Guise and Penny, 1989). Creatine kinease is released into the blood when there is muscle damage and can be used in conjunction with other indicators as a welfare measure (SCAHAW, 2002). Both dark, firm and dry (DFD) and pale, soft and exudative (PSE) meat are often related to poor welfare conditions (Tarrant, 1989).

Although plasma levels of glucocorticoids have been widely used as measures of welfare (e.g. Dantzer et al., 1983; Dantzer and Mormede 1983; Moberg, 1985), interpretation of results has several problems that have to be taken into account (Mason and Mendl, 1993; Rushen, 1986, 1991). Nevertheless, plasma levels of glucocorticoids are useful to assess welfare problems and they have provided important insights into the effects of transport upon the animals, often in combination with other measures. For example, Broom et al. (1996) studied the hormonal effects of a 15 hour road journey in sheep and showed that the major changes in plasma levels of cortisol and prolactin occurred in the first 3 hours of transport while, during the remaining 12 hours, the stimulatory effect of transport was present but small. This would suggest that welfare may be particularly poor when animals are loaded and shortly afterwards.

Heart rate can be a useful measure of the response of an animal to environmental challenge (Broom and Johnson, 1993). It has been shown, for example, that when sheep are transported by road, the movements of the vehicle caused by poor driving or bad road conditions caused an increase in heart rate that can not be explained solely in terms of increased physical activity of the animals. This result would suggest that sheep find this situation aversive. Further, transport conditions leading to an increase in heart rate also caused an increase in the incidence of DFD meat (Ruiz-de-la-Torre et al. 2001).

Acute phase proteins (APP) are proteins produced in the liver that increase or decrease in serum concentration by at least 25% in the first 7 days after tissue damage (Kushner, 1982). APP can be used to study the effects of transport and handling on the welfare of the animals. In pigs, at least two APPs (haptoglobin and Pig-MAP) increase after a 6 hour transport, whereas they do not increase after a 1 hour transport (Saco et al., 2003).

Behavioural measures can also provide useful information. Although there are many other parameters, the amount of fighting that animals show is of particular interest, as fighting may cause both injuries and stress. Fighting occurs as a consequence of social mixing rather than of transport itself, and the amount of fighting depends on the species, sex, age and transport conditions (Ruiz-de-la-Torre and Manteca, 1999; SCAHAW, 2002).

Stunning before slaughter is a legal and humanitarian requirement to ensure the insensibility of the farm animals to any noxious stimuli. Electrical stunning is one of the most widely used methods in several species, including sheep and pigs, and it consists of passing electricity through the brain to produce an instantaneous insensibility. Stunning is achieved by eliciting a tonic/clonic epileptic seizure, effectively preventing any pain stimulus from being processed in the central nervous system.

The electrical activity of the brain has been used to assess the state of sensibility of animals during slaughter. It has been shown, for example, that the physical activity of lambs after head-only electrical stunning includes one tonic phase and two clonic phases, and the recording of cortical electrical activity suggests that the animals are unconscious during the tonic phase and the first clonic phase, whereas during the second clonic phase the return of some conscious function

begins. Further, this study showed that the return of spontaneous breathing is the safest indicator that the animal is close to recovering consciousness (Velarde et al., 2002).

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PARALLEL SESSIONS E1, E2, F, G

E1 –
MICROBIAL RISK – OCCURRENCES IN SPECIES AND HUSBANDRY SYSTEMS AND RISKS OF SPREADING

ORAL PRESENTATIONS

DEAD LOSSES OF FALCONS CAUSED BY ASPERGILLOSIS

Schulz, J.[1], Sanchez, A.[2], Lierz, M.[3] and Hartung, J.[1]

[1] Institute of Animal Hygiene, Animal Welfare and Behaviour of Farm Animals, University of Veterinary Medicine Hannover, Germany; [2] Falcon Center Helvesiek, Germany
[3]Institute of Poultry Diseases, Free University of Berlin, Germany

SUMMARY

An increasing mortality of falcons was observed in a falcon breeder centre in Germany. 22 falcons which died in 2005 and 2006 were dissected and *Aspergillus fumigatus* was isolated in all cases from the lungs or air sacks. The origin of the fungi was unclear. Therefore air samples were taken in and around the premises under different meteorological conditions. *A. fumigatus* was only sporadically found in background samples, up to 400 cfu/m^3 were detected when the wind blew from a nearby mushroom factory. It was assumed that massive emission of *A. fumigatus* from this plant caused morbidity and mortality in the falcons.

Keywords: falcons, aspergillosis, *Aspergillus fumigatus,* aerial transmission, emission

INTRODUCTION

Aspergillosis is a fungal disease caused by *Aspergillus* spp. which can affect birds of prey both in freedom and in captivity. *A. fumigatus* is the predominant species isolated from infected birds of prey (1). The spores of this thermophilic mould are relatively small (2 to 4 μm), can travel and survive for longer periods in an airborne state and are deposited in lungs and air sacks birds after inhalation (2). Median concentrations in ambient air vary largely around 10 colony-forming units per cubic-meter (cfu/m^3) (3, 4). Higher concentrations in ambient air (> 100 cfu/m^3) are usually caused by emissions from sources which harbour large amounts of spores such as mouldy grains, composting materials, litter or sawdust (1, 3, 5). Birds of prey, particularly young birds, which are exposed to ambient air containing high numbers of spores from such sources, can develop an acute or chronic aspergillosis depending on the number of inhaled spores and the responsiveness of their immune system (1, 6). However, often a clear definition of the source of the spores is difficult because also naturally decomposing organic materials have to be taken into account.

This paper reports on measurements of airborne micro-organisms and spores in and around Germany's biggest falcon breeder centre in order to identify the source responsible for high fungi concentrations in the airspace of the centre and to understand the increasing mortality rates particularly of young falcons due to acute aspergillosis.

MATERIAL AND METHODS

The investigated falcon breeder centre is located in a rural region in north-west Germany housing about 500 falcons, Peregrine-, Saker- and Gyrfalcons, Red Saheen and hybrids in 189 aviaries. North of the area loosely grouped detached residential houses are situated about 100 m away. In westerly and south-westerly directions there is open field (some horses grazing) and a forest area. Close to the southern and eastern border of the falcon centre a mushroom factory was erected a few years ago.

Twenty two of 180 frozen falcons which died in 2005 and 2006 due to aspergillosis were randomly selected and dissected aseptically. Moulds were isolated with swabs from the infected lungs, air sacks and sliced aspergillomas. The isolates were incubated on Malt-agar and Czapek Dox Agar at 37 °C for 48 h. Moulds were identified by their typical growth on agar and by microscopic examination of hyphae, conidiophores and spores.

Air samples were taken simultaneously at 24 sampling days in two aviaries and at two sampling sites in the ambient air of the falcon centre from the end of March to September 2006. The outdoor sampling sites were situated close to the western and eastern border of the centre. Indoor samplings were done in aviaries close to these areas, where the lowest respectively the highest rates of morbidity were observed. At nineteen sampling days a filtration method was used and at five days the sampling was performed by means of an impactor. Fungi were sampled on polycarbonate filters as described by Saleh et al. (7) three m above the ground or with a SAS impactor (Bioscince International, Rockville, MD) in two metres height. The impactor was used for 40 seconds (flow rate 3 l/s**).** Sampled moulds were cultivated on DG-18-agar (Oxoid LTD, Basingstoke, Hampshire, England) at 37 °C for 48 h and identified as described above. Measurements were conducted at 21 different wind directions. Wind speed, wind direction, air temperature and further meteorological data were continuously measured by means of a weather station (UNIKLIMA 7, TOSS Potsdam, Germany) positioned on the top of the main building of the falcon centre 10 m above the ground.

RESULTS

Table 1 summarises the data of the dissected 22 falcons which died of aspergillosis. Only two birds were older than one year (8 and 9 years of age) belonging to pedigree birds. 19 birds (86%) five months old or younger, six females and 13 males died of acute aspergillosis. *A. fumigatus* was isolated in all cases from the respiratory tract. Pedigree species and hybrids were affected.

Table 1. Dissection results of falcons that died in 2005 and 2006 due to Aspergillosis

species, hybrids	sex*	age (month) at death	isolated pathogen	species, hybrids	sex*	age (month) at death	isolated pathogen
Falco peregrinus x Falco rusticolus	m	3	*Aspergillus fumigatus*	*Falco cherrug-rusticolus x Falco rusticolus*	f	2	*Aspergillus fumigatus*
Falco cherrug x Falco rusticolus	f	3	*Aspergillus fumigatus*	*Falco cherrug-rusticolus x Falco rusticolus*	m	2	*Aspergillus fumigatus*
Falco cherrug-rusticolus-rusticolus	m	3	*Aspergillus fumigatus*	*Falco rusticolus*	m	2	*Aspergillus fumigatus*

species, hybrids	sex*	age (month) at death	isolated pathogen	species, hybrids	sex*	age (month) at death	isolated pathogen
Falco peregrinus babylonicus	f	3	*Aspergillus fumigatus*	*Falco peregrinus*	m	96	*Aspergillus fumigatus*
Falco cherrug-rusticolus x Falco rusticolus	m	3	*Aspergillus fumigatus*	*Falco cherrug-rusticolus x Falco rusticolus*	m	2	*Aspergillus fumigatus*
Falco cherrug x Falco rusticolus	f	4	*Aspergillus fumigatus*	*Falco cherrug x Falco rusticolus*	m	3	*Aspergillus fumigatus*
Falco cherrug x Falco rusticolus	m	4	*Aspergillus fumigatus*	*Falco rusticolus*	f	108	*Aspergillus fumigatus*
Falco cherrug-rusticolus-rusticolus x F.rusticolus	m	3	*Aspergillus fumigatus*	*Falco cherrug x Falco rusticolus*	m	2	*Aspergillus fumigatus*
Falco cherrug-rusticolus-rusticolus	f	3	*Aspergillus fumigatus*	*Falco cherrug-rusticolus x Falco rusticolus*	m	5	*Aspergillus fumigatus*
Falco rusticolus	f	9	*Aspergillus fumigatus*	*Falco cherrug-rusticolus x Falco rusticolus*	m	5	*Aspergillus fumigatus*
Falco cherrug-rusticolus x Falco rusticolus	f	2	*Aspergillus fumigatus*	*Falco cherrug-rusticolus x Falco rusticolus*	m	3	*Aspergillus fumigatus*

* m = male, f = female

Figure 1 presents the relationship between the wind direction and the amounts of *A. fumigatus* found at the outdoor sampling sites. Wind speed varied between 1 and 2.5 m/s during the measurements.

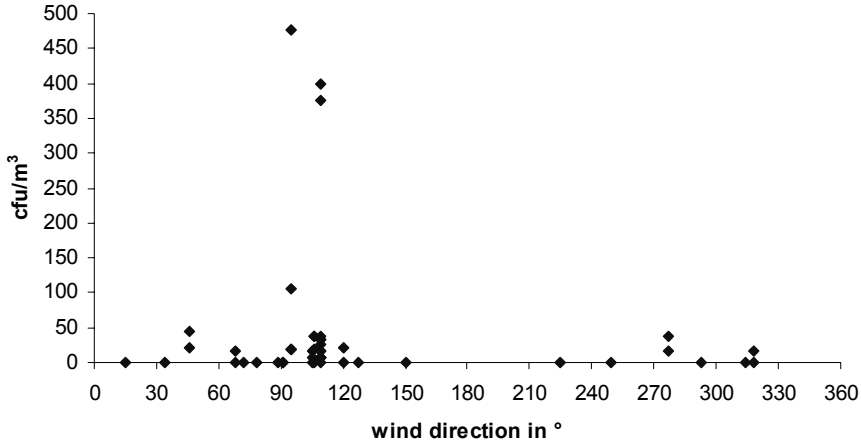

Figure 1. *Aspergillus fumigatus* concentrations in the ambient air of the falcon centre at two sampling places in relation to the wind direction. 0° = North, 90° = East, 180 = South, 270° = West

Highest concentrations of *A. fumigatus* are observed when the wind comes from east-southeast directions. At all other wind directions the concentrations do not exceed 45 cfu/m^3. No data are available when the wind came (very rarely) from the south. The results indicate that there is a potential emission source of *A. fumigatus* located in east south-east directions of the falcon centre.

The measurements inside the aviaries revealed *A. fumigatus* concentrations between 15 and 42 cfu/m^3. However, no fungi could be detected at 17 sampling days out of 24.

DISCUSSION

The results of this study show that the heavy losses in the falcon breeding centre are mainly caused by acute and chronic aspergillosis. *A. fumigatus* which is seen as the predominant causative agent of aspergillosis in falcons (1, 8) was found in all fallen birds. 86% of the 22 out of 180 randomly selected birds were only 2 to 5 months old. This is typical for acute aspergillosis which particularly affects young birds when an overwhelming number of spores are inhaled (1, 6). The highest concentrations of *A. fumigatus* were regulatory found in the air space of the falcon breeder centre when the wind blew from eastern to south-eastern directions. In all other wind directions the airborne concentration of fungi was not detectable or below 45 cfu/m³. Although there is no clear definition of the infective dose for *A. fumigatus* to cause aspergillosis it seems likely that the periodically occurring massive emissions from the mushroom factory may have triggered the disease. The periodical character of the emissions (due to the wind direction) is supported by the results from the measurements inside the aviaries. Although the shelter walls of the aviaries reduce the number of fungi penetrating into the air space of the aviaries in some cases up to 45 cfu/m³ of *A. fumigatus* could be found inside.

The conditions of a captive environment may stress birds in general and can make them more susceptible to disease (8). Also poor husbandry, management and hygiene can increase the likelihood of infection (6). This may be in general terms also true for this holding. However, during inspections of all bird shelters regularly over a period of nearly a year no indication of poor management conditions and hygiene could be found. The managers made every effort to minimize the environmental stressors. The aviaries were regularly cleaned and experienced permanently employed staff was taking care of the animals. The good status of the aviaries is also confirmed by the low concentrations of fungi in the indoor air space when the wind is not coming from eastern directions.

The fact that the heavy losses of falcons caused by *A. fumigatus* infections began after the erection of the mushroom factory and increased steadily with the expansion of the mushroom production also supports the hypothesis that the mushroom factory may be the main contributor to the aspergillosis in the falcon centre. Mushroom plants use wood chips, peat and sawdust and other biological materials as growing substrate. Such materials regularly contain high amounts of *A. fumigatus* spores and also mycotoxins (10, 11). When the material is mixed high amounts of these compounds are emitted into the air and can travel easily 100m and more because of there minute dimensions. Windy and dry conditions support a far reaching distribution.

CONCLUSION

It is assumed that the dead losses due to aspergillosis observed in a falcon breeder centre were caused by strong emissions of *A. fumigatus* from a mushroom factory situated about 100 m upwind. It is recommended that planning authorities should carefully consider "safe distances" between mushroom factories to sensitive animal holding facilities such as falcon farms. This may be also useful in respect to residential areas.

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IDENTIFICATION OF AIRBORNE FUNGI FROM INDOOR AIR OF CHICKEN HOUSES BY RAPD-PCR

Chai, T.[1], Wang, Y.*[1.2], Lu, G.[2], Song, C.[3], Sun, Y.[4] and Qi, C.[1]

[1] College of Animal Science and Veterinary Medicine, Shandong Agricultural University, Tai'an 271018, PR. China; [2] College of Life Science, Dalian Nationalities University, Dalian 116600, China; [3] Shandong Agricultural Administration College, Jinan 250100; [4] China Agricultural University, Yantai, 264670, China

ABSTRACT

The objectives of this study were to examine the airborne fungal concentrations, size and compositions as well as the dominant genera in chicken houses. Aerosol fungi were collected from chicken house indoor with Andersen-6 stage air sampler and cultured in RBC media. Species were identified by morphological characteristics and RAPD. The concentrations of aerosol fungi in chicken houses ranged from $1.8–3.0\times10^3$ CFU/m^3 air. 89 *Fusarium* isolates collected from the chicken houses were characterized by RAPD. Out of 30 primers, five primers reproducibly generated polymorphic patterns for fungal species of a test panel, with 74 characteristic fragments. These primers were then used in RAPD assay of 91 isolates. RAPD profiles were clustered with UPGMA algorithm. This analysis helped clarify *Fusarium* isolates that were difficult to produce conidia for morphological identification.

Keywords: chicken house; airborne fungi; RAPD; genetic distance; *Fusarium*

INTRODUCTION

Fusarium species are common dominant mould in animal farm environment [1□2□3□4]. Traditionally, these species are identified by their morphological and physiological characteristics especially at vegetative stage. These methods are simple and convenient. However, a taxonomic system based on morphology is often factitious. It does not necessarily reflect evolutionary history. Morphology of the genus *Fusarium* is highly variable and extremely complex. In addition, it is difficult or sometimes impossible to obtain the reproductive stages. Even well-trained mycologist may not be able to diagnose one thirds of *Fusarium* isolates to species level [5]. Thus, Identification of *Fusarium* species has been considered the most difficult among fungi [6]. Application of random amplified polymorphic DNA (RAPD) technology in identification of *Fusarium* may be fast and accurate.

In recent years, many studies have focused on fungal aerosol concentrations in hospital, residential and public environment [7, 8, 9, 10]. A literature search indicates that fungal aerosol in animal husbandry environment has not yet been considered as occupational hazard. Here we report the concentration and identification of airborne *Fusarium* species in poultry house with RAPD approach. This study is necessary for evaluation of occupational hazardous exposure for workers in animal husbandry environment.

1. MATERIALS AND METHODS

1.1 Sample Collection

Air samples were collected between November, 2003 and December, 2005 from three chicken farms (Tai'an Manzhuang Chicken Farm, Zaozhuang Mengzhuang Township Chicken Farm and Liuling Tiekuang Chicken Farm) in Shandong Province. Collection was performed using an Anderson 6-stage air sampler [11, 24, 25] at a speed of 28.3 L/min and 50 cm above ground. Each air sample was collected in 2–4 min depending on the environment. For each farm, samples were taken three times alternated by one week. In each time three to five samples were collected.

1.2 Fungal Culture and Identification

Fungi were cultured with RBC media [12] in alternatively lighted incubator for 3–7 d at 25°C. Colonies were counted according to their morphology under Olympus stereoscope. Pure cultures were established and preserved. Fungal isolates were tested in SNA (saccharose nirenberg agar), PDA (potato dextrose agar) and PSA (potato sucrose agar) plates. *Fusarium* species were identified according to their colony characteristics and reproductive structure in reference to the systems of Booth (1971) [13], Gerlach & Nirenberg (1982) [14], Nelson (1983) [15] and Joffe (1986) [16].

1.3 DNA Extraction

The fungal isolates were cultured at 25°C for 6–7 d in 0.5 mL liquid medium (glucose yeast medium) in 1.5 mL Eppendorf tube. Three cultures were made for each isolate. *Aspergillus niger* and *Trichoderma viride* were also cultured as controls. Genomic DNA was isolated as described [17] and DNA concentration was determined by electrophoresis in 1% agarose gel and imaged by GDS 8000 (UVP, US).

1.4 RAPD-PCR

1.4.1 Materials 100 bp DNA ladder, 10×PCR buffer, 10×ExTaq Buffer, dNTP (25 mmol/dm^3 each), ExTaq DNA Polymerase (5U/μL) were purchased from Takara Biotechnology (Dalian) Co., Ltd. Random primers (10bp in size) were purchased from Shanghai Sangon. PCR amplification was performed with PE2400 or PE2700 thermal cycler (ABI, USA).

1.4.2 RAPD Reaction Mixture The volume of each reaction was 25μl. The reaction mixture contains: (1) random primer (table 1) 0.2 μmol/dm^3; (2) dNTP 0.2 μmol/dm^3; (3) PCR buffer 2μL (10 mmol/dm^3 Tris-HCl, pH9.0, 50 mmol/dm^3 KCl, 1.5 mmol/dm^3 $MgCl_2$); (4) genomic DNA 1μL (5 ng DNA); (5) *Taq* DNA polymerase 0.2μL (1U); and (6) highly purified water 18.8 μL.

1.4.3 Thermal Cycle ① Pre-denaturating at 94°C for 200 sec. ② 40 cycles of denaturation at 94°C for 1 min, annealing at 36°C for 1 min, and extension at 72°C for 2 min. ③ Final extension at 72°C for 5 min. Reaction products were preserved at 5°C.

1.4.4 RAPD Product Visualization RAPD products were mixed with loading dye (phenol blue-EDTA-glycerol). The product mixtures were separated by electrophoresis in 2% agarose gel for 1–2 h at 10 V/cm. Gel images were recorded and analyzed with UVP system GDS 8000.

1.4.5 Screening for RAPD Primers Primers were suspended in TE buffer in a working concentration of 20 μM. They were tested against a panel of 6 *Fusarium* species with the condition described in section 1.4.1 through 1.4.4. The panel contains isolate No. 61 (*F. ventricosum*), 15 (*F. morniliforme*), 57 (*F. oxysporum*), 60 (*F. graminearum*), 59 (*F. poae*) and 39 (*F. equiseti*). Five primers that were able to produce 4–10 bands for each of the panel species were selected for the analysis of 91 isolates. Reactions were duplicated for each isolate.

1.4.6 Data Analysis The presence of a band in an isolate was represented by "1", while the absence was represented by "0". Distance between two samples were calculated by equation of D=1–2Nxy/Nx+Ny, where Nxy is the sum of bands in the two samples; Nx is the number of bands in sample x and Ny is the number of bands in sample y. Distance matrix was produced using unweighted pair-group method with arithmetic mean (UPGMA) algorithm and a dendrogram was generated by EPCLUST software.

2. RESULTS

2.1.1 Fungal Concentration Airborne fungal concentrations in closed chicken house were between $1.8\sim3.0\times10^3$ CFU/m^3. The chicken density was 5.9~10.2 chicken/m^2. Temperature variation was within 3°C. Humidity was 47% ~ 73%.

2.1.2 Airborne *Fusarium* 150 isolates of airborne *Fusarium* were finally obtained from the three farms. Of which 89 strains were picked randomly for RAPD assay. They belong to the following species: *F. moniliforme, F. moniliforme* var. *intermedium*, *F. morniliforme* var. *subglutinans*, *F. oxysporum*, *F. equiseti*, *F. solani*, *F. semitectum*, *F. graminearum*, *F. ventricosum*, *F. poae*, *F. avenaceum*, *F. nivale*.

2.2 Primer Screening

30 primers were screened using DNA samples extracted from 6 fungal strains. Five primers (*e. g.* P15, P17, P19, P24 and P29) were able to produce highly polymorphic bands. The number of bands produced varied from 5 to 15. They were selected for the analysis of the 89 isolates and 2 control strains.

2.3 RAPD Patterns

The five selected primers were used to amplify DNA from 89 isolates and 2 control strains. Reactions were performed under the same condition and with the same thermal cycler. PCR products were separated by agarose gel electrophoresis and analyzed with high resolution gel documentation equipment. PCR product sizes were obtained by comparing with the molecular weight standard. The product patterns were similar among isolates belonging to the same species while the patterns exhibit greater variation among species. Each species displayed a unique characteristic profile (Fig 1).

2.4 Pattern Matrix Generation

A matrix was generated according to the presence or absence of RAPD-PCR fragments in basic pairs (bp). The bands within the following molecular size (bp) are marked as presence: 1950–

2250(r1), 1750–1950(r2), 1550–1750(r3), 1350–1550(r4), 1150–1350(r5), 950–1150(r6), 850–950(r7), 750–850(r8), 650–750(r9), 550–650(r10), 450–550(r11), 350–450(r12), 250–350(r13), 150–250(r14), 100(r15). The presence of a band within the above range was assigned a value '1', while the absence of a band was assigned a value '0'. 91 isolates were tested with the five primers. 73 bands were obtained. A band pattern matrix was generated (data not shown here).

2.5 Genetic Distance

Isolates were clustered using UPGMA to calculate genetic distance. A dendrogram was generated from the distance matrix (Fig. 2). The distances among isolates are small. When threshold distance was set at 0.62, the 91 isolates were divided into 11 groups.

3. DISCUSSION

3.1 Plentiful diversity of aerosol fungi was found in the three tested chicken farms. The most frequent aerosol fungi are *Aspergillus,* some of which are opportunistic pathogens. For example, *A. fumigatus* and *A. terreus* were recorded to infect human and animals. Animal tests have shown that some *Aspergillus* species (e. g. *A. flavus*, *A. parasiticus*, *A. versicolor*) may produce aflatoxins that induce tumor or reduce white blood cells. The other frequent fungi in the farms include *Penicillium, Alternaria, Acremonium* and *Fusarium.* Some *Penicillium* species may clinically infect human beings who have been affected by leukaemia or lymphoma, or infect brain or lung and produce ochratoxins. Some *Alternaria* species may clinically cause skin infection such as hypersensitivity, pneumonitis or asthma. Some species of *Alternaria* may produce mycotoxins that induce oesophageal cancer. *Acremonium* may cause chromomycosis or phaeohyphomycosis. They commonly infect brain or skin. It is surprisingly found that *Fusarium* is a very common genus in these tested chicken houses. *Fusarium* species are very common in agricultural environment and some well-known for producing various mycotoxins. A few *Fusarium* species may induce skin or cornea ulcers, even in rare case being associated with cancer [12, 18–22]. The various fungal species may show certain virulence to the animals. It is necessary to further the study of their pathogenic ability to chickens.

3.2 When threshold is set at 0.62, the tested *Fusarium* isolates can be differentiated into species that can be identified by morphological method. However, the five RAPD primers were unable to distinguish among *F. moniliforme F. moniliforme* var. *subglutinans* and *F. moniliforme* var. *intermedium* because of their close relationship. 75% isolates of the same species were clustered into the same group. Nearly 25% isolates were not clustered to groups in which they were supposed to be. This explains the variability of *Fusarium* species from molecular biology point of view. Some isolates (*e.g.* 27 and 28) are extremely close. They might belong to the same species of different isolates. The difference in collecting sites may result in different genetic distance of the same *Fusarium* species, such as Dalian isolate and Tai'an isolate of *F. moniliforme.* Its genetic distance between airborne isolates and isolates from wheat grains is relatively far-away, suggesting that the complicated environmental factors may cause variation of *Fusarium* species. This result is consistent with the instability of *F. moniliforme* [23].

F. graminearum did not produce conidia even cultured on SNA plate, which makes identification by morphology difficult. An isolate (No. 43) which is difficult identified did not form conidia on either PDA or PSA. Its purple pigment-producing colony indicates that it is

Fusarium. By clustering analysis of RAPD patterns, it can be confidently assigned to *F. graminearum.* Using RAPD marker, some difficult isolates can be identified. It is concluded that RAPD assay could provide as an effective tool for the identification of suspected fungal isolates.

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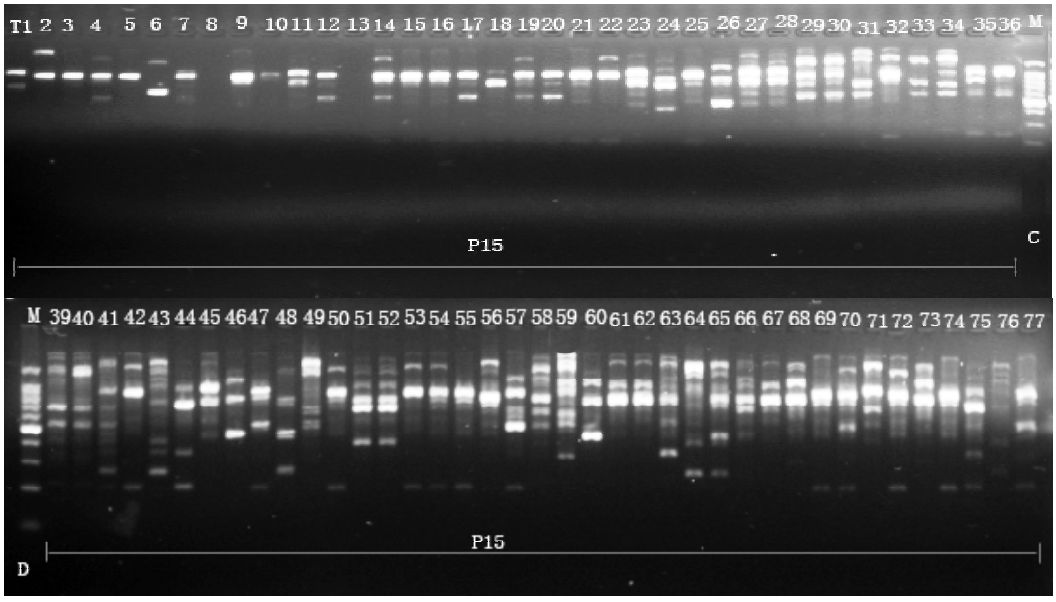
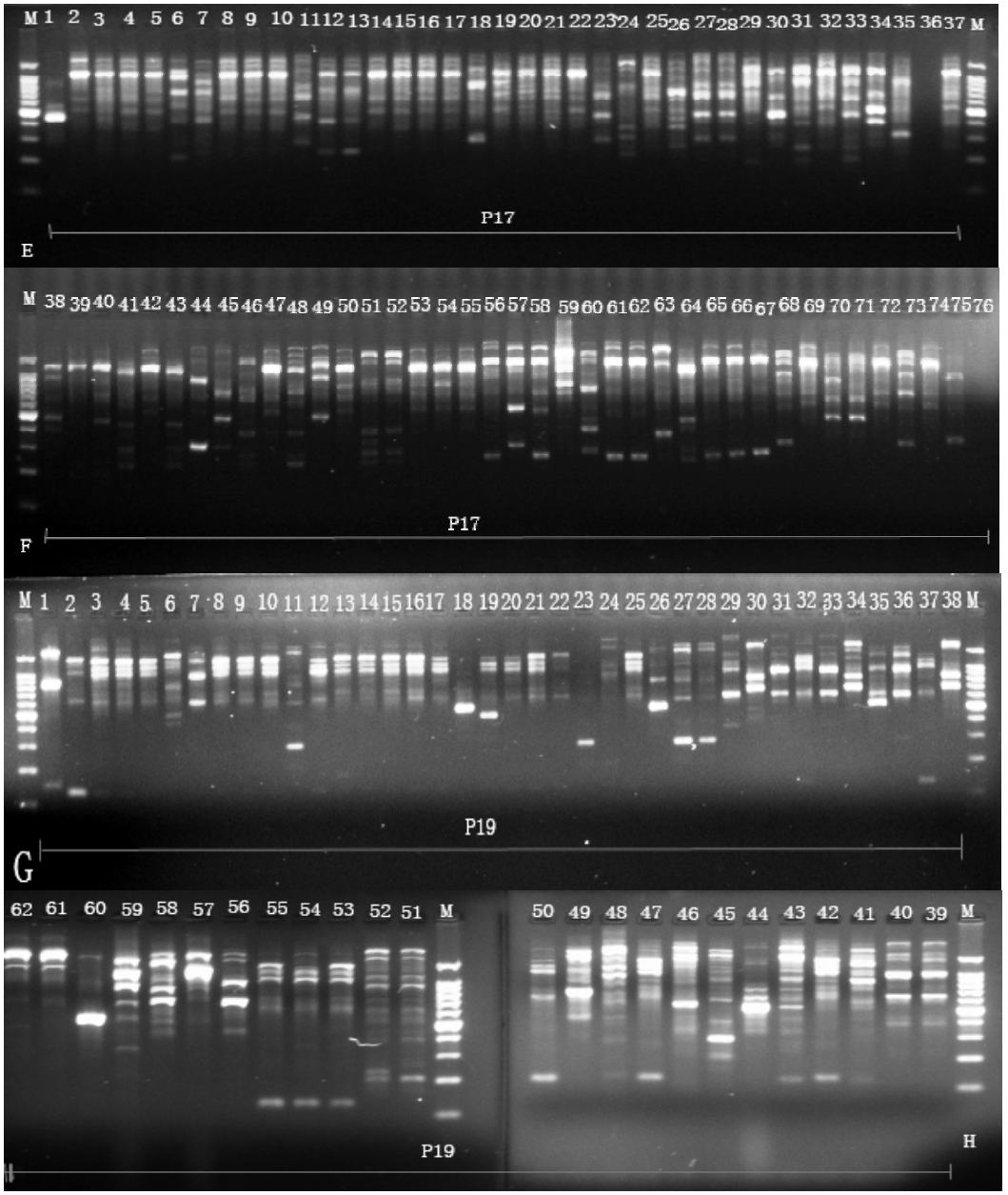
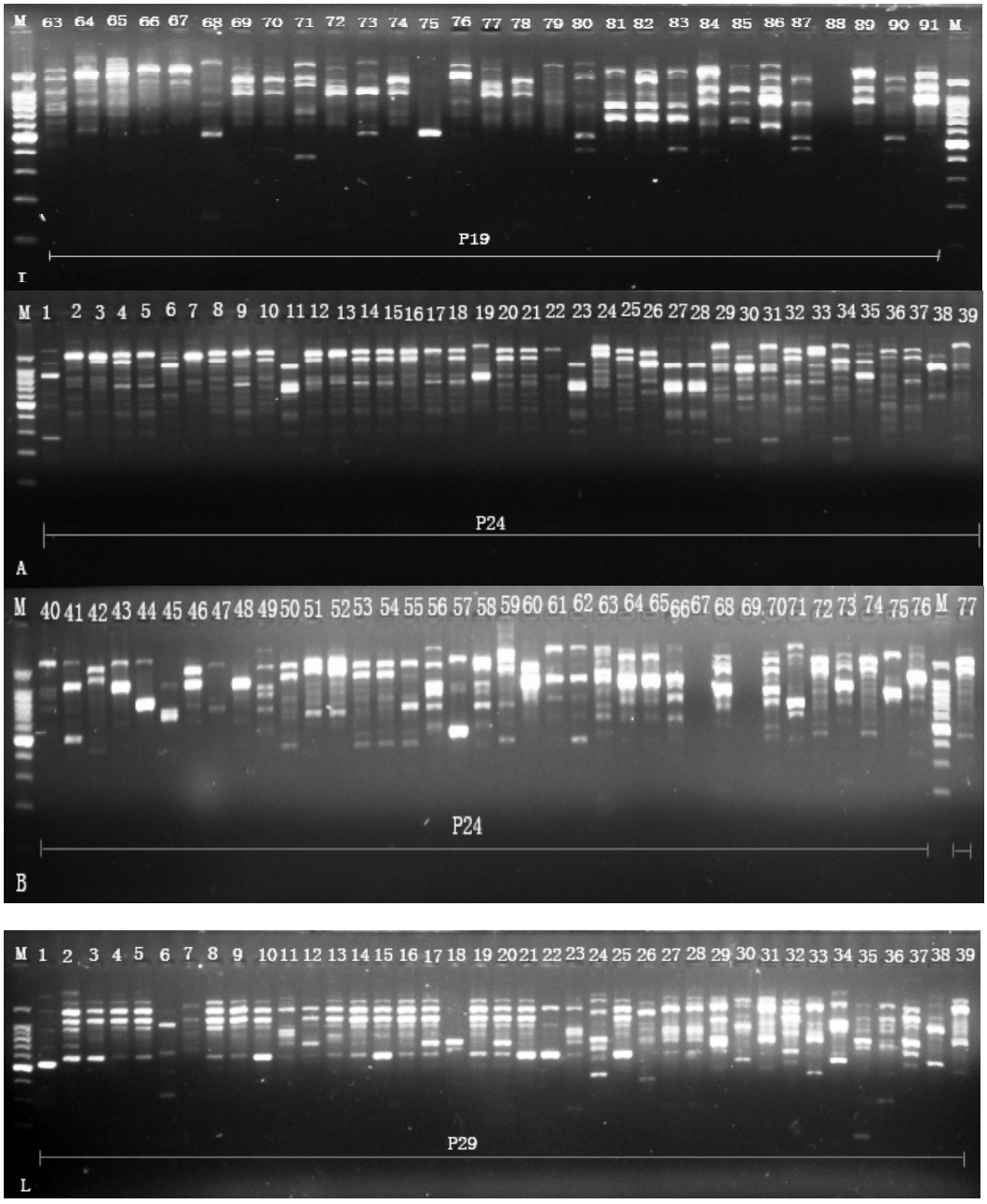
Table 1. Oligonucleotide sequences of 30 primers for RAPD-PCR

code	No.	Sequence	code	No.	sequence	code	No.	sequence
P1	S23	agtcagccac	P11	S22	tgccgagctg	P21	S130	ggaagcttgg
P2	S125	ccgaattccc	P12	S24	aatcgggctg	P22	S326	gtgccgttca
P3	S329	caccccagtc	P13	S30	gtgatcgcag	P23	S324	aggctgtgct
P4	S25	aggggtcttg	P14	S28	gtgacgtagg	P24	S129	ccaagcttcc
P5	S26	ggtccctgac	P15	S330	ccgacaaacc	P25	S322	cctacgggga
P6	S124	ggtgatcagg	P16	S21	caggcccttc	P26	S126	gggaattcgg
P7	S27	gaaacgggtg	P17	S29	gggtaacgcc	P27	S328	gggtgggtaa
P8	S123	cctgatcacc	P18	S327	ccaggaggac	P28	S325	tcccatgctg
P9	S127	ccgatatccc	P19	S122	gaggatccct	P29	S121	acggatcctg
P10	S321	tctgtgccac	P20	S323	cagcaccgca	P30	S128	gggatatcgg




M 1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 20 21 22 23 24 25 26 27 28 29 30 31 32 33 34 35 36 37 M
P17
E
M 38 39 40 41 42 43 44 45 46 47 48 49 50 51 52 53 54 55 56 57 58 59 60 61 62 63 64 65 66 67 68 69 70 71 72 73 74 75 76
P17
F
M 1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 20 21 22 23 24 25 26 27 28 29 30 31 32 33 34 35 36 37 38 M
P19
G
62 61 60 59 58 57 56 55 54 53 52 51 M
50 49 48 47 46 45 44 43 42 41 40 39 M
P19
H


M 63 64 65 66 67 68 69 70 71 72 73 74 75 76 77 78 79 80 81 82 83 84 85 86 87 88 89 90 91 M
P19
I
M 1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 20 21 22 23 24 25 26 27 28 29 30 31 32 33 34 35 36 37 38 39
P24
A
M 40 41 42 43 44 45 46 47 48 49 50 51 52 53 54 55 56 57 58 59 60 61 62 63 64 65 66 67 68 69 70 71 72 73 74 75 76 M 77
P24
B
M 1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 20 21 22 23 24 25 26 27 28 29 30 31 32 33 34 35 36 37 38 39
P29
L

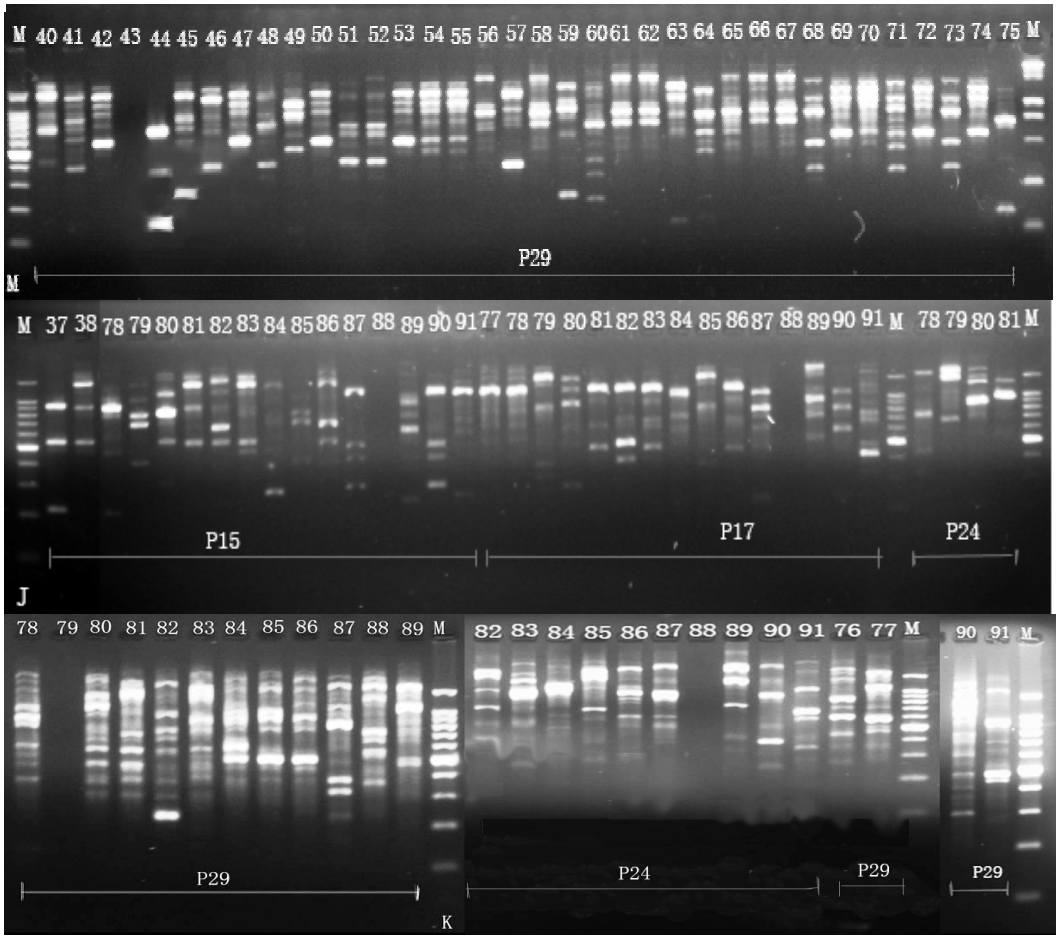


Figure 1. RAPD profiles for 91 strains using 5 optimized RAPD primers

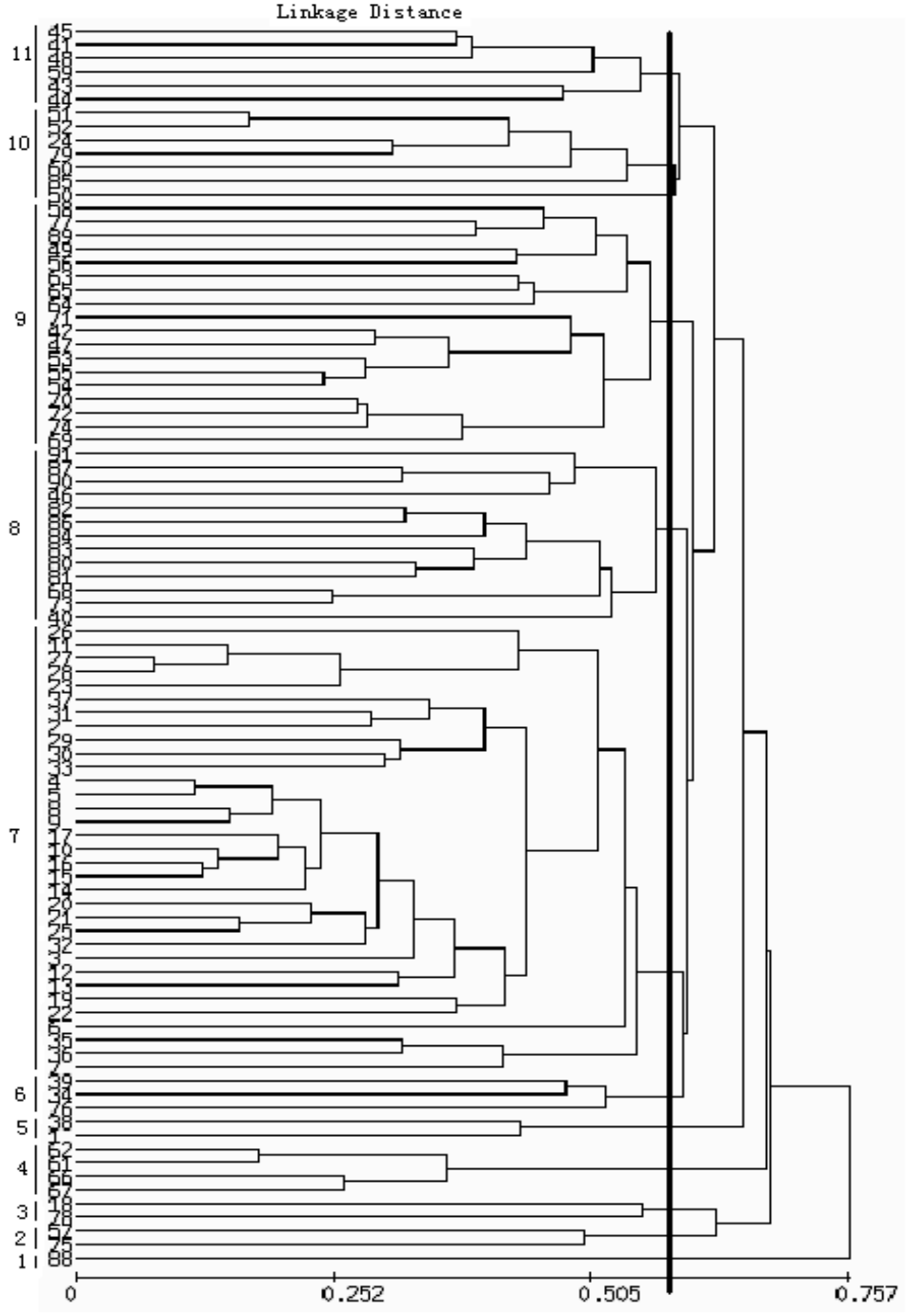


Figure 2. Dendrogram for 91 *Fusarium* strains based on RAPD pattern

WOOD SHAVINGS AND BIOCOMPOST AS BEDDING MATERIAL IN HORSE STABLES TO ENSURE AIR QUALITY DEMANDS – THE AGONY OF CHOICE?

Seedorf, J.[1] and Hartung, J.[2]

[1] University of Applied Sciences, Oldenburger Landstr. 24, 49090 Osnabrück, Germany;
[2] Institute of Animal Hygiene, Animal Welfare and Behaviour of Farm Animals, University of Veterinary Medicine Hannover, Foundation, Bünteweg 17p, 30559 Hannover; Germany

SUMMARY

Objective: The susceptibility and reaction of stabled horses to inhaled airborne pollutants is often associated with bedding materials, which have a poor hygienic status. In a field study we evaluated a new biocompost bedding material for horse stables with respect to its impact on air hygiene and compared the results to those from a reference trial with wood shavings.

Methods: The study was conducted in a naturally ventilated stable. Ammonia and thermophilic actinomycetes as air hygiene parameters were measured 24 hours a day for seven days with each bedding type.

Results: During the monitoring period wood shavings were associated with mean ammonia concentrations of 12.6 ppm, while biocompost caused only 5.1 ppm. On the other hand, the concentrations of airborne thermophilic actinomycetes were highest with biocompost (14,822 vs. 84 colony forming units per m³).

Conclusions: This investigation clearly shows that potential advantages and disadvantages of new bedding materials have to be weighed very carefully. To ensure the well being of horses, any new bedding material must be tested very carefully before it is introduced to the market.

Keywords: horse, biocompost, wood shavings, bedding material, air hygiene

INTRODUCTION

Recurrent airway obstruction (RAO; chronic obstructive pulmonary disease, COPD; heaves) in horses is linked closely to aerial environmental factors including noxious gases and bioaerosols, which may be highly concentrated in the air inhaled by animals kept indoors (Clarke 1987). The susceptibility and reaction of stabled horses to inhaled bioaerosols is often associated with mouldy bedding material and foodstuff containing fungal spores and actinomycetes. In the aetiology of chronic respiratory diseases the quality of raw bedding materials and type of management can be of great relevance, because differences in the ability of these materials to release particulates can be often observed. While straw is a major source of released particles, wood shavings emit only negligible or moderate amounts of particles. Alternative bedding materials for horses such as biocompost are being sought in order to optimise indoor environmental conditions. Biocompost is made of plant wastes, disintegrated, and fermented by aerobic microbes and supplemented with peat because of its water binding capacity.

The present study evaluated such a biocompost bedding material for horse stables with respect to its impact on air hygiene and compared the results to those from a reference trial with wood shavings. The whole study is comprehensively documented and published by Seedorf et al. (2007).

MATERIAL AND METHODS

The study was conducted in a naturally ventilated stable with four pens occupied by one horse each. The basic microclimate between pens was characterised by online electronic measurement of the temperature and relative humidity of the air within the pens. The concentrations (ppm) of the aerial gases ammonia (NH_3) was determined continuously by photoacoustic infrared spectroscopy.

A set of IOM (Institute of Occupational Medicine, Edinburgh, UK) samplers was equipped with polycarbonate filters (pore size 0.8 µm) to sample airborne thermophilic actinomycetes in the pens. Exposed filters were shaken gently with sterile isotonic NaCl solution to dissolve the collected microorganisms. Afterwards the basic suspension was diluted and an aliquot of the dilutions was inoculated on glycerol arginine agar. The aerobic incubation temperature was 50°C for thermophilic actinomycetes. In accordance with their specific growth potencies, growing colonies were counted after 14 days of incubation. The findings are expressed as colony forming units (CFU) per m^3 air. Measurements were also made outdoors. Reference measurements of temperature, relative humidity and total bacteria were made to record environmental influences on indoor conditions during the trials. I

Data were recorded 24 h a day during all 7 sampling days within the 2-week trial with each bedding material tested. Each measurement sequence started at 06.00 h and stopped automatically at 06.00 h the following day. The next sequence was initiated 24 h later and so on. The continuously measured data on temperature, relative humidity and gas concentrations were averaged per day and box. The calculated mean values were then expressed in relation to a maximum available sample size of n = 28 (4 boxes x 7 days). Due to the cumulated sampling procedure over 24 h, the theoretical sampling size for airborne thermophilic actinomycetes was also 28. Since there may be natural fluctuations in the numbers of colony forming units during the cultivation of microorganisms, 3 replicates were carried out per box and day, to give an ideal sampling size of 84. As the Shapiro-Wilks test showed that the data sets were not distributed normally, medians, and minimum and maximum values are presented. The Mann-Whitney U test was used for statistical comparison of the corresponding data for the same tested factor on the basis of the sampling sizes for each trial.

RESULTS

There was an indoor median temperature difference of 2.9°C between the test phases with wood shavings (21.5°C) and biocompost (18.6°C). In contrast to the significant temperature difference, the relative humidity was similar with wood shavings (66.8%) and biocompost (69.6%). During the 7-day monitoring periods the average ammonia concentrations scarcely exceeded 5 ppm in the stable with biocompost, while it was nearly 13 ppm with wood shavings. According to these data the difference was significant ($p<0.001$). Minimum and maximum values ranged from 1.6 ppm to 9.1 ppm (biocompost) and from 1.8 ppm to 22.9 ppm (wood shavings).

The median concentration of thermophilic actinomycetes was 14,822 CFU/m³ with biocompost and 84 CFU/m³ with wood shavings ($p<0.001$). A peak concentration of 113,426 CFU/m³ during the biocompost trial corresponded to a maximum value of 503 CFU/m³ during the wood shavings test phase.

The median outdoor values were 15.8°C and 73.0% during the wood shavings trial and 12.0°C and 77.0% during the biocompost trial. The relative magnitude of bacterial accumulation in the stable was assessed by outdoor measurements conducted once a day over the entire trial period. Concentrations were similar in both surveys, with 750 CFU/m³ outdoors during the wood shavings trial and 875 CFU/m³ during the biocompost trial ($P>0.05$).

DISCUSSION AND CONCLUSIONS

Animal welfare considerations have led to recommendations that ammonia concentrations in stables should not exceed 10 ppm (Anon 1995). In this study, NH_3 was nearly 13 ppm with wood shavings and with biocompost only 5.1 ppm, maximum <10 ppm. Biocompost can efficiently absorb urine and the relatively high water content enhances the shift from volatile ammonia to water-bound ammonium. Because the biocompost was supplemented with small amounts of peat, both its water (urine) holding and ammonia binding properties were improved (Airaksinen et al. 2001).

Certain important health-related properties of biocompost can be attributed to its production conditions. For example, thermal energy is generated when organic plant waste rots. The increase in temperature due to aerobic fermentation may have a sanitising effect, but favour the growth of thermophilic microorganisms. Therefore, an accumulation of bacterial types such as thermophilic actinomycetes can be expected. From this point of view the potential of biocompost to release actinomycetes is a serious problem, because these microbes may induce sensitisation of the airways, ultimately leading to COPD (Mair and Derksen 2000).

In conclusion, the biocompost bedding material tested in this study seems to be an alternative to other common materials; among its positive hygienic and animal welfare properties these are low concentrations of airborne ammonia, for example. However, its enrichment with substantial amounts of thermophilic actinomycetes, represents a potential health threat, because human and animal activities in the stable cause the release and accumulation of inhalable actinomycetes and complementary agents (e.g. moulds) into the stable air, potentially initiating and maintaining respiratory disorders in susceptible individuals due to combined effects. In consideration of these aspects, biocompost tested here cannot be recommended as bedding material for horses in stables. Furthermore, this report also stresses the necessity of testing any new bedding material product before it is introduced to the market and weighing very carefully its advantages and disadvantages to ensure the well-being of horses.

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ANALYSIS OF FAECAL SAMPLES FROM SCRAPIE-INFECTED SHEEP AND BSE-INFECTED CATTLE FOR PRP^{RES}

Hunsinger, B., Hilss, K. and Böhm, R.

Institut für Umwelt- und Tierhygiene sowie Tiermedizin mit Tierklinik, Universität Hohenheim, Germany

SUMMARY

Faecal samples from sheep and cattle, artificially infected with Scrapie or BSE, were examined for the presence of prion protein (PrP^{res}).

Sheep faecal samples, collected between day 1 and 5, and 37 days and 157 days post infection, and cattle faecal samples collected after 4, 7 and 8 months post infection were examined. A method was developed including several steps of chemical and physical enrichment and Western Blotting for the sensitive detection of prion proteins in faeces.

PrP^{res} was detectable in artificially spiked sheep and cattle faecal samples but not in any of the examined faeces samples of the infection experiments.

Keywords: TSE-agents, prion protein, faeces, enrichment, infection route, risk assessment

ORIGINAL ASPECTS OF THE RESEARCH

So far there have been no findings on the possible excretion of TSE-agents in the faeces of TSE-infected animals nor has it been shown that faeces from these animals are contagious. These results have to be questioned due to the fact that only small quantities of faecal material had been analyzed.

In the present study faecal samples from sheep and cattle, artificially infected with Scrapie or BSE, were examined for the presence of prion protein (PrP^{res}).

The aim of the study was furthermore to identify possible infection routes of TSE-agents and to minimize or completely eliminate the risk of infectivity for the environment.

METHODS

A total of 36 sheep and 6 cattle faecal samples were tested in two independent trials.

The collected faecal samples originated from infection experiments of three groups of sheep, which were orally infected with 4 g of homogenized brain material from Scrapie-infected sheep and two groups of cattle orally infected with BSE which had been fed brain material from BSE-positive cows. Sheep faecal samples collected between day 1 and 5, and 37 days and 157 days post infection, and cattle faecal samples collected 4, 7 and 8 months post infection were examined.

Ten grams of the faecal samples were dissolved in 30 ml Phosphate buffered saline. Three serial dilution steps were prepared. For isolation and enrichment of low amounts of prion protein in these faecal samples and the dilution steps a reisolation method was developed including several steps of chemical and physical enrichment.

To degrade rough particles in the faecal samples an acid hydrolysis using formic acid was performed. Dehydol 980, a detergent, was then added, and after an incubation time of 10 min at room temperature the samples were centrifuged for 5 min at 1,000 x g. The supernatant was transferred to another tube and L-Sarcosin was added to both, supernatant and pellet. Following a 30 min incubation time at room temperature the samples were centrifuged for 4 min at 2,000 x g. The pellets were discarded and the supernatants pooled. A third centrifugation for 10 min at 2,000 x g was carried out following 40 min incubation at 3°C with bovine serum albumin and ice-cold ethanol.

The supernatant was discarded and the remaining pellet was resuspended in Tris/HCl buffer with L-Sarcosin.

Following a proteinase K digestion (5 µg proteinase K in 30 ml for 45 min at 37°C) to unmask the epitope the enriched samples were tested for PrP^{res} using Western Blot and immunodetection techniques.

For the separation of the precipitated proteins SDS-polyacrylamide gels (PAGE) were used. The concentration of the resolving gel was 15.0%.

9.0 µl of reducing loading buffer (RotiLoad) was added to the samples and heated for 5 min at 95°C. After the following centrifugation step for 2 min at 14,000 x g to remove undissolved proteins, the supernatant was separated using SDS-PAGE for 60 min at 150 V (elecrophoresis buffer: 1.0 g SDS, 6.0 g Tris, 28.8 g Glycine in 1 l distilled water).

Scrapie and BSE specimens from the FLI (Friedrich Löffler Institut, Tübingen) served as a positive control. The molecular weight marker was purchased from Santa Cruz (Cruz Marker™Molecular Weight Standards, sc-2035) and the protein marker from Peqlab (peqGold Protein-Marker IV prestained).

After electrophoresis the gel was removed and following a washing step in TBST (Tris-Buffered Saline Tween-20, 7.88 g Tris-HCl, 8.76 g NaCl, 1 ml Tween®20 in 1 l distilled water) for 10 min at 30 rpm (Rocking Shaker) incubated in transfer buffer (100.0 ml methanol, 2.9 g Tris, 14.6 g Glycine in 1 l distilled water) at room temperature.

The blotting paper and the PVDF (Immobilon-P-Polyvinylidene Difluoride) membrane which was moistened with 100% methanol were equilibrated in transfer buffer. After blotting at 80 mA for 1 hour the PVDF membrane was washed three times for 5 min each with TBST and incubated with blocking solution (3.0 g skim milk in 100 ml TBST) for 1 hour at 4°C on a rocking shaker at 30 rpm. Then it was washed again with TBST three times for 5 min for each washing step.

For the detection of prion protein an enzyme immunoassay (EIA) was performed.

To detect scrapie agent in the sheep samples the first antibody (POM 1, mouse anti-prion protein, FLI Tübingen) was diluted in blocking solution at a ration of 1:2,000 and the membrane was incubated with 10 ml of the POM1 antibody solution overnight at 4°C. The PVDF membrane was washed three times for 5 min each with TBST, incubated for 1 hour at room temperature with the second antibody (goat anti-mouse IgG-HRP sc-2031) which was diluted in blocking solution at a ratio of 1:1,000 and washed three times with TBST, once for 5 min with TBS.

Western blotting Luminol reagent solution was used to visualize the PrP^{res} and the western blot was documented by photography (Hyperfilm ECL, Amersham Biosciences, 5 min).

To detect BSE-agent in the cattle samples EIA was performed as described above but monoclonal antibody L42 (Friedrich-Löffler-Institut Tübingen) in a dilution 1:500 was used as the primary antibody.

RESULTS

PrP^{res} was detectable in artificially spiked sheep and cattle faecal samples with a detection limit of 10^4 infectious units. There was no PrP^{res} detectable in any of the examined faecal samples of the infection experiments (table 1) although the enrichment factor of the applied reisolation method is approx. 400 (figure 1).

Table 1. Detection of PrP^{res} in faecal samples of orally infected sheep and cattle

	Sheep faecal samples: days post infection							Cattle faecal samples: months post infection		
	1	2	3	4	5	37	157	4	7	8
Detection PrP^{res}	–	–	–	–	–	–	–	–	–	–

– : PrP^{res} not detectable

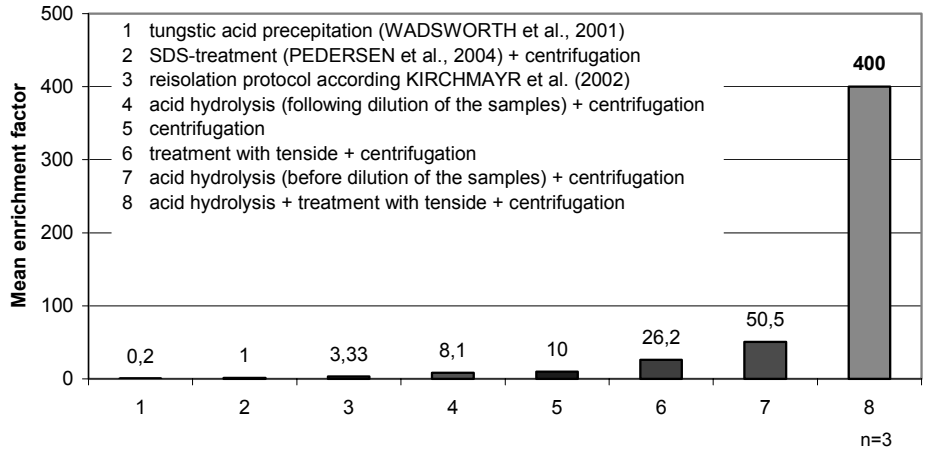

Figure 1. Comparison of different reisolation protocols for the detection of PrP^{res} in faecal samples

CONCLUSIONS

The animals tested were all infected orally. We therefore expected a positive detection of PrP^{res} in the first days after oral infection with a high infectious dose and even in a later phase of the infection.

The results indicate that if PrP^{res} is excreted at all, only very low amounts (vestiges) of PrP^{res} might be present in the faeces which are not detectable by the reisolation and detection method applied. It is possible that PrP^{res} is adsorbed, resorbed, or degraded in the gastrointestinal tract, or it may be excreted at a later phase during the TSE pathogenesis. In further studies sheep faecal samples > 6 months and cattle faecal samples > 8 months post infection will be analyzed using the technique described and a bioassay will be performed in parallel to detect potential residual infectivity in faeces.

SALMONELLA INFECTION LEVEL IN DANISH INDOOR AND OUTDOOR PIG PRODUCTION SYSTEMS MEASURED BY ANTIBODIES IN MEAT JUICE AND FAECAL SHEDDING ON-FARM AND AT SLAUGHTER

Bonde, M. and Sørensen, J.T.

University of Aarhus, Faculty of Agricultural Sciences, Department of Animal Health, Welfare and Nutrition, P.O. Box 50, DK-8830 Tjele, Denmark

SUMMARY

The prevalence of Salmonella shedding was compared in 34 organic, conventional outdoor and indoor pig herds. Individual faecal and meat juice samples from 30–50 pigs per herd were analysed for presence of Salmonella, and Salmonella antibodies, respectively. We found low levels of Salmonella shedding on farm and at slaughter in organic and conventional outdoor herds compared to indoor pigs. Overall 5,5% of the pigs were seropositive. The serological test result was associated with Salmonella shedding at slaughter in pigs from conventional systems, but not in organic pigs. The duration of transport did not affect the risk of Salmonella shedding.

Keywords: fattening pig, production systems, Salmonella, faecal shedding, organic pigs

INTRODUCTION

Pork and pork products are recognised as one of the major sources of human salmonellosis (Lo Fo Wong et al. 2002, Wegener and Baggesen 1996). Pigs in outdoor production systems benefit from a low animal density, and access to outdoor area, and organic pig production furthermore differs from conventional production in terms of feeding, weaning age, and use of preventive medication (Bonde and Sørensen 2004). It is therefore likely that the risk of Salmonella is different in organic, outdoor, and indoor pig production. The level of Salmonella shedding at slaughter might differ between the production systems, caused by differences in the level of resistance to the pathogen, which may be due to the immune system based disease resistance and/or components of the husbandry systems affecting disease development and pathogen shedding (Zheng et al, 2007).

Stege et al. (2000) found a herd level association between high seroprevalence and presence of Salmonella in faecal samples. Jensen et al. (2004) observed a higher prevalence of Salmonella antibodies in outdoor than indoor pig production systems, and Hald et al. (1999) also reported that the proportion of seropositive pigs tended to be higher in conventional outdoor production systems compared to pigs from either organic or indoor production systems. On the other hand Meyer et al. (2005) reported that conventional slaughter pigs were more likely to be seropositive than organic pigs. The presence of antibodies indicates that the pig has been exposed to challenge by the enteric pathogen at some stage of its development.

A number of stress factors related to the routine management in a pig herd may increase the risk of infection, as stress can induce carriers to shed Salmonella at a higher rate and increase the susceptibility of Salmonella-free pigs to infection (Mulder, 1995). Transport of pigs to the abattoir causes significant stress to the animals, which can trigger an increase in shedding (Lo Fo Wong et

al. 2002), and duration of transport and lairage may also affect the level of Salmonella shedding at slaughter (Morgan et al., 1987). It is therefore essential to compare the faecal shedding before and after transport to the abattoir, when assessing the risk of pathogen transfer into the food chain.

The objective of this survey was to investigate the effect of different pig production systems with indoor or outdoor rearing, and the effect of transport duration, on the potential pathogen transfer risk into the food chain from Salmonella in pig faeces. Further we evaluated the predictive value of the serological test result in relation to Salmonella shedding at pig level.

MATERIALS AND METHODS

Eleven organic, 12 conventional outdoor and 11 indoor fattening pig herds were included in the survey. The median yearly production of slaughter pigs in the herds amounted to 1300 pigs in the organic herds, 2750 pigs in the conventional outdoor herds and 1935 pigs in the indoor herds. Sows and suckling piglets were kept outdoors on pasture in organic as well as conventional outdoor herds. The organic fattening pigs from weaning at seven to eight weeks of age to slaughter were housed in deep litter pens with access to an outdoor area with concrete floor, with a total space allowance (indoor and outdoor) of min. 2,30 m^2 per 100-kg pig. Eight of the organic herds fattened their own piglets, while three herds bought 30-kg pigs from other organic herds. Two of the organic herds kept the growing pigs on pasture until they weighed 60–80 kg. The organic pigs were fed organic feed and were provided with roughage. Preventative medication with antibiotics was not applied in the herds. Conventional outdoor pigs from weaning at four to five weeks of age to slaughter were housed in deep litter pens with access to an outdoor area with concrete floor, with a total space allowance (indoor and outdoor) of min. 1,20 m^2 per 100-kg pig. Six conventional outdoor herds fattened their own piglets, while the remaining six herds bought 30-kg pigs from other outdoor herds. The conventional outdoor pigs got conventional feed without roughage supply. Preventative medication with antibiotics was not applied in the herds. The indoor pigs were housed in indoor pens without access to outdoor areas and roughage. The indoor pigs were typically weaned at three to four weeks of age, kept in two-climate pens in the sow herd until 30 kg live weight, and then moved to pens with either solid concrete or slatted floor, and mostly with a space allowance of less than 0,75 m^2 per slaughter pig. Three indoor herds fattened their own piglets, while the remaining eight herds bought 30-kg pigs from indoor sow herds.

During a one-year period faecal samples were collected in each herd from 3–5 batches of 10 randomly chosen and individually marked pigs 1–7 days before slaughter, and the animals were clinically examined. The examination assessed the animals visually in accordance with a clinical protocol focusing on body condition, general appearance, lesions, skin and haircoat, locomotive disorders, diarrhoea, constipation, and respiratory symptoms. Meat juice samples and samples of caecal content from the individual pigs were further collected at the abattoir. Faecal and caecal samples were cooled and sent to the laboratory to be analysed qualitatively for density of enteric Salmonella using the modified NMKL method. Positive samples were further analysed semi-quantitatively, and the cultures were serotyped. A meat sample from each pig was frozen, and meat juice (harvested after thawing) was examined for specific antibodies against Salmonella enterica using an indirect enzyme-linked immunosorbent assay (ELISA) (Nielsen et al., 1998). The ELISA combined several S. enterica O-antigens, and allowed detection of antibody response after a variety of different S. enterica serovar infections. Samples with an OD%>10 were considered seropositive.

Information about duration of transport to slaughter was collected from 155 batches of pigs (50 organic, 58 conventional outdoor, and 47 indoor batches).

Qualitative bacteriological and serological data at pig level were analysed by log-linear models (Using the SAS Proc GENMOD), taking herd and batch into account. Serological test response, Salmonella shedding on-farm and Salmonella shedding at the abattoir were all assumed binomial distributed with logit as the link function. The models were:

1. Serological test response (positive/negative) = system (organic, conventional outdoor, indoor) + any clinical symptoms (yes/no)
2. Salmonella shedding on-farm (positive/negative) = system (organic, conventional outdoor, indoor) + serological test response (positive/negative) + any clinical symptoms (yes/no)
3. Salmonella shedding at slaughter (positive/negative) = system (organic, conventional outdoor, indoor) + serological test response (positive/negative) + Salmonella shedding on-farm (positive/negative) + duration of transport (<1 hour, 1–3 hours, > 3 hours) + system* serological test response

Differences in transport duration between systems were analysed in SAS by Proc GLM, and differences in clinical symptoms between systems were analysed in SAS by Proc GENMOD.

RESULTS

The prevalence of Salmonella in the different production systems is illustrated in Table 1. Overall 5,5% of the pigs were seropositive with no significant differences between systems (P=0,11). The overall prevalence of Salmonella in faecal samples from pigs on-farm was 0,87%; the systems were significantly different (P<0,0001). Neither of the clinical parameters, e.g. diarrhoea, constipation or poor body condition, was associated with Salmonella shedding on farm. The prevalence of Salmonella shedding at slaughter was 2,2% of the sampled pigs, with a significant difference between systems (P<0,05). We obtained paired samples from 1556 pigs.

Table 1. The prevalence of Salmonella in pigs from 34 Danish herds with different production systems

	Serology meat juice		Salmonella shedding on-farm		Salmonella shedding at the abattoir	
System	N	% positive animals	N	% positive animals	N	% positive animals
Organic	539	7,2	593	0,17	537	1,9
Conventional outdoor	561	4,6	616	0,16	555	1,1
Indoor	465	4,5	600	2,7	474	4,0

Shedding of Salmonella on-farm was significantly predicting shedding at slaughter (P<0,0001), but differences in transport had no effect on Salmonella shedding at slaughter. Seropositive organic pigs were less likely to be shedding Salmonella at slaughter (0%) than seropositive pigs from the conventional indoor and outdoor production systems (10–12%) (P<0,01) (Figure 1). Late Salmonella infections occurred in all production systems with 0,5–4% of the seronegative pigs shedding Salmonella at slaughter. In the conventional systems an antibody positive test result was a significant predictor of Salmonella shedding at slaughter.

The duration of transport is illustrated in Figure 2. The mean durations of transport to slaughter were 175,3 min (organic pigs), 128,6 min (conventional outdoor pigs) and 96,8 min (indoor pigs) (P<0,0001).

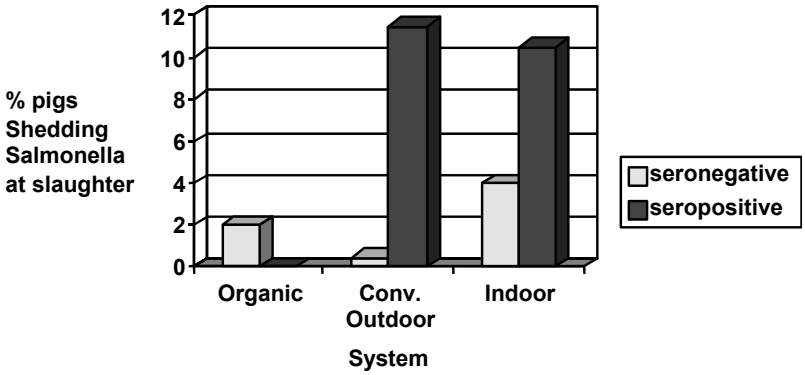

Figure 1. Prevalence of Salmonella shedding at slaughter in antibody positive and antibody negative pigs from 34 pig herds with different production systems

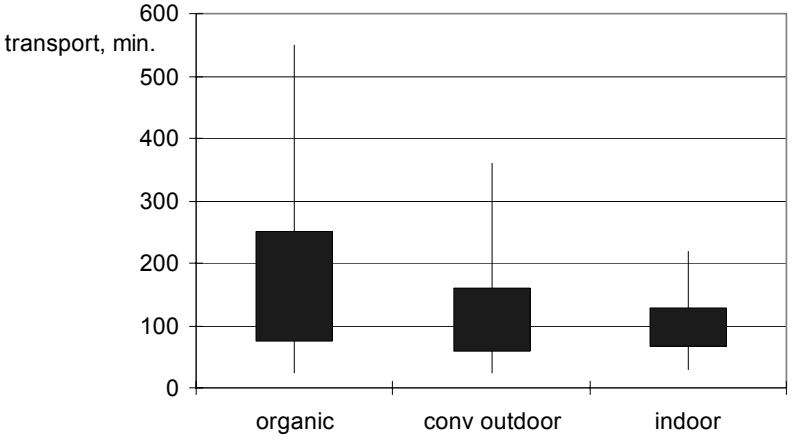

Figure 2. Duration of transport to the abattoir: min, max, 25% and 75% quartiles of the transport duration in minutes for the three pig production systems.

DISCUSSION AND CONCLUSION

In the survey we found similar Salmonella seroprevalences in outdoor and indoor systems. The result is consistent with Ledergerber et al. (2003), who compared the level of Salmonella infections in conventional and animal-friendly farms in Switzerland. In contrast to this, Jensen et al. (2004) found a higher seroprevalence of Salmonella in outdoor than indoor pig production system, and in a survey by Hald et al. (1999) the proportion of pigs from organic production

systems testing positive for antibodies against Salmonella was not different from pigs reared in indoor production systems, while the proportion of antibody positive pigs tended to be higher in conventional outdoor production systems. On the other hand, Meyer et al. (2005) reported that conventional slaughter pigs were significantly more likely to be seropositive than organic pigs.

The prevalence of Salmonella shedding in pigs from outdoor systems was significantly less than in indoor herds. Baggesen et al. (1996) found an overall prevalence of Salmonella shedding at slaughter of 6,2%, which is higher than the overall prevalence of 2,2% in this survey, and also of the prevalence for indoor pigs: 4,0% in this survey. There is a ten-year difference between the two surveys, so the apparent difference probably is the effect of the current Salmonella control programme in Denmark aiming to minimise the risk of Salmonella in slaughter pigs (Mousing et al., 1997). The lack of association between Salmonella shedding and clinical symptoms is in agreement with Stege et al. (2000) reporting predominantly subclinical salmonellosis in Danish finishing pigs. The low levels of Salmonella shedding in organic and outdoor pigs suggest that pigs from low input systems may be more resistant to the pathogen, or may encounter the infection earlier in life so they have cleaned themselves from infection at time of slaughter. The poor association between seropositivity and shedding of Salmonella in organic pigs at slaughter indicates that a serological test might be better suited to conventional than organic herds as a means to identify individual pigs more likely to shed Salmonella.

The differences in transport duration recognised in this survey did not affect the risk of Salmonella shedding at slaughter. The transport distances in general were rather small, and it is likely that a notable effect of transport relies on more substantial differences in transport time.

ACKNOWLEDGEMENTS

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DISCLAIMER

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BACTERIOLOGICAL AND VIROLOGICAL INVESTIGATIONS ON THE USE OF QUICKLIME FOR THE DISINFECTION OF EGG SHELLS AND EGG SCRAPS

Philipp, W., Marschang, R.E. and Böhm, R.

OBJECTIVES

The disposal of eggshells and egg scraps is regulated by EU by-law No. 1774/002. In Germany, regulation is by the law and the by-law on the disposal of animal by-products (Tierische Nebenprodukte Beseitigungsgesetz (TierNebG) and the Tierische Nebenprodukte Beseitigungsverordnung (TierNebV)). The use of egg shells and egg scraps is also regulated by the new fertilizer by-law (Düngemittelverordnung (DüMV)), according to which egg shells can be used as lime fertilizer following hygienization. The objective of this study was therefore to determine if and under what conditions (amount and time) quicklime (CaO) can be used to inactivate salmonella, Newcastle disease virus (NDV), and the enterovirus ECBO-Virus in egg shells and egg scraps, allowing the use of the treated egg material as fertilizer. The second was how we can doe a process validation by using quicklime for the disinfection of egg shells and egg scraps.

SUMMARY

This study describes the validation of a treatment process for egg shells and egg scraps with quick lime using salmonella, an enterovirus (ECBO virus) and Newcastle disease virus (NDV) according to EU-regulation No. 1774/2002. The hygienization of egg shell material is influenced by the water content of the egg shell material, the age of the quicklime used and the difficulties inherent in sufficiently mixing the quicklime with the egg shell material. Despite these factors, our study showed that treatment of egg shell material with 50 kg quicklime per ton for 7 days is sufficient to reduce possible risks due to bacteria and viruses to below a justifiable limit.

Keywords: EU-regulation No. 1774/2002, disinfection, egg shells, salmonella, ECBO virus, Newcastle disease virus (NDV), hygenization, quicklime, validation, fertilizer

METHODS

A suspension of *Salmonella typhimurium* (approximately 10^6 cfu/g egg shell material) was homogenously mixed into egg shell material in laboratory and practical tests. Various amounts of granulated and ground quicklime (15–100 kg/t egg shell material) were added. An enterovirus (ECBO virus) was added on germ carriers in both laboratory and practical tests. In the main tests, ECBO virus was added to the egg shell material on germ carriers (HOFERER, 2001). For the germ carriers, the virus suspension was diluted 1:10 in phosphate loading buffer (88.9 parts KH_2PO_4 9,073 g/l and 11.1 parts $Na_2HPO_4xH_2O$ 11,87 g/l). The virus suspension was then adsorbed onto a virosorb membrane (Cuno, Waldbronn). The membranes were then enclosed in

0,01 µm polycarbonatmembrane (Infiltec GmbH, Speyer). Tests with NDV were only carried out in the laboratory and the virus was added by mixing eggs that were infected with NDV to the egg shell mixture before treatment with quicklime. In addition, the natural content of aerobic bacteria, Enterobacteriaceae, *E. coli,* and *Enterococcus faecalis* of the egg shell material before and after the addition of quicklime as well as the pH and the dry matter content were determined.

RESULTS

Tenacity of salmonella

In the first practical test ground quicklime (white fine lime) was used in concentrations of 15, 25 and 50 kg per ton in egg shells and egg scraps. In this test 15 kg CaO/t material were sufficient to inactivate the test salmonella within 3 and 14 h (Tab. 1). In the second practical test only granulated quicklime was used in concentrations of 25 and 50 kg per ton. After 3 h the test salmonella were not inactivated. However, no salmonella were detected after 14 h (Tab. 1). In a third practical test we examined the effect of concentrations of 15 and 25 kg granulated and ground quicklime on the test salmonella. In the first and in the second practical test 15 kg ground quicklime were sufficient to inactivate salmonella within 3 h. Granulated quicklime was insufficient to inactivate salmonella within 14 h (Tab. 1). The results of the third practical test were comparable with the first test and showed that 15 kg ground quicklime were sufficient to inactivate salmonella within 14 h. With 25 kg CaO the time for the inactivation of salmonella was only 3 hours (Tab.1). In two laboratory tests quicklime (ground) in contents of 25 to 100 kg/ton egg shells and egg scraps were used in contact times between 1, 3 and 7 days. Table 1 shows the results (excluding the tests with 100 kg quicklime) of the laboratory tests. In the first laboratory test even 75 kg of quicklime/ton did not inactivate salmonella within 7 days. The second laboratory test showed that 25 kg quicklime was enough to inactivate salmonella within 7 days. A quantitative detection of salmonella with concentrations of 25, 50, 75 and 100 kg quicklime after a contact time of one hour was impossible.

Table 1. Results of the studies on the inactivation of salmonella in egg shell material with quicklime (3 practical and 2 laboratory tests)

	Quicklime concentration per ton egg shell material					
Practical test	**15kg ground**	**15 kg granulated**	**25 kg ground**	**25 kg granulated**	**50 kg ground**	**50 kg granulated**
1. test	yes	n.d.	yes	n.d.	yes	n.d.
3 h	–		–		–	
14 h	–		–		–	
2. test	n.d.	n.d.	n.d.	yes	n.d.	yes
3 h				+		+
14 h				–		–
3. test	yes	yes	yes	yes	n.d.	n.d.
3 h	+	+	–	+		
14 h	–.	–	–	+		

Table 1. Continuation

Laboratory test	25kg qual.	25 kg quant.	50 kg qual.	50 kg quant.	75 kg qual.	75 kg quant.
1. test						
1 d	+	–	+	–	+	–
3 d	/	/	/	–	/	/
7 d	+	–	+ [1)]	–	+	+ [1)]
2. test	**25 kg qual.**	**25 kg quant.**	**50 kg qual.**	**50 kg quant.**	**75 kg qual.**	**75 kg quant.**
1 d	+	–	–	–	–	–
3 d	+	–	–	–	–	–
7 d	–	–	–	–	–	–

[1)] = reduction of about 6 log 10
yes = appropriate lime contents used in the tests
n.d. = not done
\+ = salmonella positive
– = salmonella negative
/ = no investigations accomplished
qual. = qualitative results
quant. = quantitative results

Tenacity of other bacteria

Table 2 shows the results of the amount of salmonella (S), Enterobacteriaceae (EBA), *E. coli (EC), Enterococcus faecalis* (ECF) and aerobic bacteria (AEB) in untreated eggshells and egg scraps from the third practical test.

Table 2. Natural bacterial content of the egg shell material in the third practical test (cfu/g)

Sample	S Qual.	S Quant.	EBA	EC	ECF	AEB	pH-value	DM
I	O:4/O:9	$4{,}3 \cdot 10^2$	$2{,}3 \cdot 10^7$	$1{,}5 \cdot 10^3$	$2{,}3 \cdot 10^5$	$5{,}9 \cdot 10^8$	8,37	67,9%

DM = Dry matter

In the egg shell material salmonella could found in concentrations of $4{,}3 \cdot 10^2$ cfu/g substrate (serogroups O:4 und O:9). While *E. coli* was counted in concentrations of $1.5 \cdot 10^3$ cfu/g and *Enterococcus faecalis* with $2{,}3 \cdot 10^5$ cfu/g, the count of Enterocbacteriaceae was $2.3 \cdot 10^7$ and the number of aerobic bacteria was $5.9 \cdot 10^8$ cfu/g (Tab. 2). Table 3 shows the results after the application of quicklime (concentration of quicklime used 25 kg/m^3; granulated, ground).

Table 3. Bacterial contents in the egg shell material after treatment with two different concentrations of quicklime (cfu/g).

Concentration CaO/t	Contact-time	Salm. Qual.	Salm. Quant.	EBA	EC	ECF	AEB	pH
Ground								
25 kg	3 h	–	–	–	–	–	$1{,}7 \cdot 10^3$	12,86
25 kg	14 h	–	–	–	–	$3{,}6 \cdot 10^0$	$1{,}1 \cdot 10^3$	12,78
Granulated								
25 kg	3 h	+	$4{,}3 \cdot 10^3$	$9{,}3 \cdot 10^3$	–	$2{,}3 \cdot 10^3$	$2{,}9 \cdot 10^5$	12,78
25 kg	14 h	+	$1{,}5 \cdot 10^2$	$9{,}3 \cdot 10^2$	–	$9{,}3 \cdot 10^1$	$1{,}2 \cdot 10^5$	12,76

With the use of 25 kg ground quicklime neither Enterobacteriaceae (EBA, *E. coli* (EC) and *Enterococcus faecalis* (ECF) could be detected. When 25 kg granulated quicklime was used, the values of these microorganisms varied between > 10^1 and 10^3 cfu/g substrate (Tab.3). The pH value was > 12 in all of the samples.

Virological results

Preliminary tests showed that the titre of ECBO virus on germ carriers decreased more slowly than that of NDV. Therefore, in the main test we used only ECBO virus on germ carrier, because this procedure was easier. The tenacity of ECBO virus in egg shells and egg scraps with a dry matter content of about 40% is shown in table 4.

Table 4. ECBO virus in 50 kg egg shell and egg scrap substrate (titre in $\log_{10}$ $TCID_{50}$/ml)

Quicklime concentration*	**conditions**	**0**	**2 h**	**24 h**	**48 h**	**5 d**	**7 d**
Controls:	Refrigerator	**7,25**					**6,5**
	DM			**6,25**			**5,75**
	40%			**6,25**			**6,25**
25 kg	DM		**2,5**	**2,5**	≤2,5	≤1,5	≤1,5
	40%		**2,5**	**2,5**	≤1,5	≤1,5	≤2,5
50 kg	DM		**3,0**	**2,5**	≤2,5	≤2,5	≤2,5
	40%		**2,25**	**3,5**	≤2,5	≤1,5	≤2,5

* Pro 1000 kg DM: original dry matter content; 40%; Water content adjusted to 40%

The titre of ECBO virus on the germ carriers at the beginning of the tests was 7,25 $\log_{10}$ $TCID_{50}$/ml. After 2 hours the titre was reduced by approx. 4 $\log_{10}$ steps in all tests. All viruses were inactivated after 48 hours.

CONCLUSIONS

Considering the variable conditions encountered in practice (changing water contents, different age of the materials, difficulties in mixing the quicklime with the egg shells and egg scraps) a concentration of ground quicklime of 50 kg per ton material and a storage time of 7 days is generally recommended. If these conditions are met, epidemic-hygienic residual risk is justifiable for the utilization of the limewashed egg shells and egg scraps as fertilizer in agriculture.

Recommendations for reducing salmonella by about 6 $\log_{10}$ cfu

Kind of lime	**amount/ton**	**time (days)**
CaO ground	30–50 kg	at least 3 d
CaO granulated	30–50 kg	at least 3–7 d

Recommendations for inactivating ECBO virus + Newcastle disease virus (NDV)

Kind of lime	amount/ton	contact time (days)
CaO ground	50 kg	at least 3 – 7 d

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SPATIAL DISTRIBUTION OF PRIMARY OUTBREAKS OF HIGHLY PATHOGENIC AVIAN INFLUENZA IN NIGERIA

Ekong, P.S.[1]., Olugasa, B.O.[2]., Oyetunde, I.L.[1]., Waziri, N.E.[1] and Joannis, T.M.[1]

[1] *Epidemiology Unit, Central Diagnostic Laboratory, National Veterinary Research Institute, Vom, Nigeria, E-mail: piusekg@yahoo.com;* [2] *Department of Veterinary Public Health and Preventive Medicine, University of Ibadan, Ibadan, Nigeria*

ABSTRACT

The geographic coordinates of 116 out of 166 poultry farms (69.9%) with confirm Highly Pathogenic Avian Influenza (HPAI) virus by the National Veterinary Research Institute (NVRI) in Nigeria, out of 629 premises that were screened within 30 States and the Federal Capital Territory (FCT) were used to design a spatial model of the spread of the virus within Nigeria from January 1, 2006 to January 31, 2007. The results showed that 19 of 36 States (52%) of Nigeria were affected with the virus. Movement of personnel from one infected farm to an uninfected farm, transfer of infected birds and contaminated equipment were the main sources of spread of the virus. Thus, there is need to improve the animal health services in Nigeria to attain efficient control of HPAI.

INTRODUCTION

Highly pathogenic avian influenza is a devastating disease of poultry, associated with high death rates that disrupt poultry production and trade (*6, 5*). The first outbreak of the disease in Africa was reported in Nigeria in January 2006. The outbreak occurred among commercial poultry flock in Kaduna, Plateau and Kano States, all in northern Nigeria. The outbreak later spread to the southern part of the country affecting 13 States within a period of 7 weeks (*3*). Since then, seven other African countries, namely, Egypt, Niger, Cameroon, Burkina Faso, Sudan, Cote d'Ivoire and Djibouti have reported infection and disease outbreaks (*1*).

Nigeria's first Highly Pathogenic Avian influenza (HPAI) outbreak occurred on January 16, 2006 and was caused by the H5N1 strain of the virus. The confirmation was based on laboratory tests carried out at the National Veterinary Research Institute (NVRI) in Vom, Plateau State, Nigeria and validated by the OIE Reference Laboratory for HPAI in Padova, Italy. Following the subsequent spread of the disease across poultry flocks in northern and southern Nigeria, all samples from suspected cases requiring laboratory test were confirmed at the National Veterinary Research Institute (NVRI) in Vom, Plateau State, Nigeria. It has therefore become possible to provide consistent reports of the disease emerging trend across the country. More than 100 poultry farms have since been confirmed positive. The farms are located in different States of Nigeria.

The aim of this study was to describe the spatial distribution and risk of spread of HPAI among poultry in Nigeria based on laboratory-confirmed records at the NVRI between January 2006 and January 2007.

MATERIALS AND METHODS

This study covered the period from January 16, 2006 to January 31, 2007, which corresponds to the primary outbreak of HPAI in Nigeria. The data were pooled for the entire time series from the NVRI. The coordinates of farms affected were collected using Personal Digital Assistant (PDA) running Global Positioning System (GPS). Non-spatial data were collected through case reports that accompanied the specimens for diagnosis. Every non infected farm within 5km and 10km radii of an infected farm was inventoried for spatial analysis on risk of spread. Spatial and non-spatial data gathered were added to ESRI ArcGIS Desktop for mapping.

RESULTS

The NVRI laboratory received a total of 570 samples from poultry farms and local chicken communities in 29 states and the Federal Capital Territory (FCT), Abuja, between January and December 2006. 134 (23.5%) were confirmed positive for H5N1. The positive cases originated from 17 states and the FCT. In January 2007, 32 (54.2%) of the 59 samples tested were confirmed positive for H5N1 in poultry.

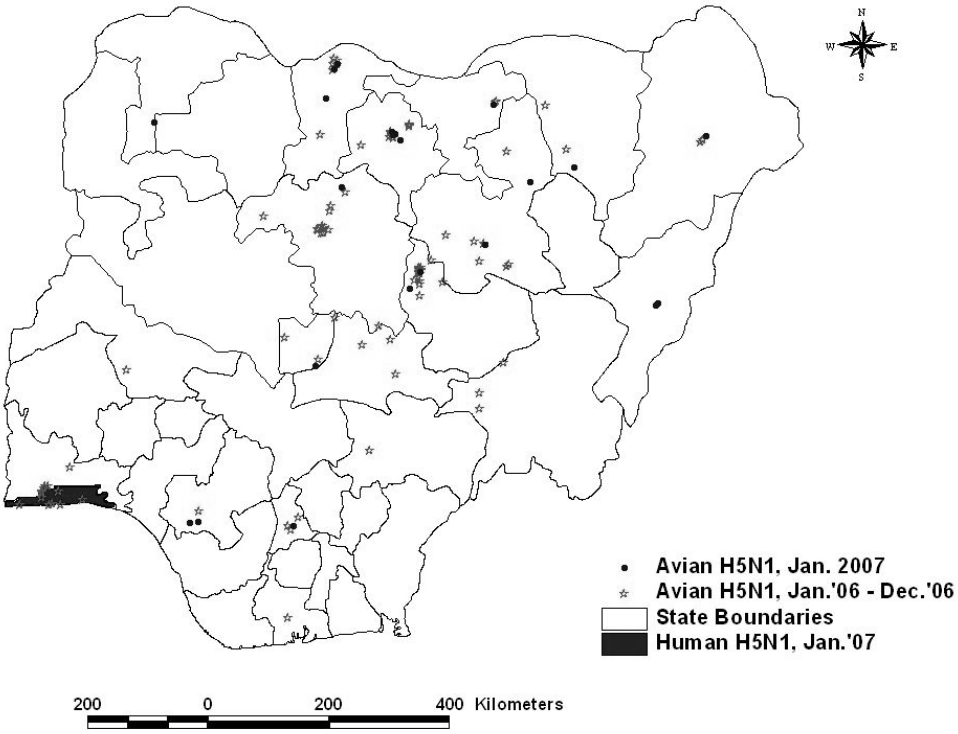

Figure1. Spatial distribution of HPAI outbreaks in poultry in Nigeria from January 2006 to January 2007 and location of the single human case in south-western Nigeria

In all, H5N1 outbreak was confirmed in 166 (26.4%) premises from 629 submitted from 19 states and the FCT from January 1, 2006 to January 31, 2007. The farms were distributed in various Nigerian states as shown in the Figure 1 above. There are 36 States in Nigeria and one Federal Capital Territory.

Figure 2 shows the epidemic curve of HPAI in Nigeria from January 2006 to January 2007, as well as a curve of the total number of states infected. The latter curve gives an indication of the spatial extent of the disease in the country, while the former indicates the magnitude of the outbreak.

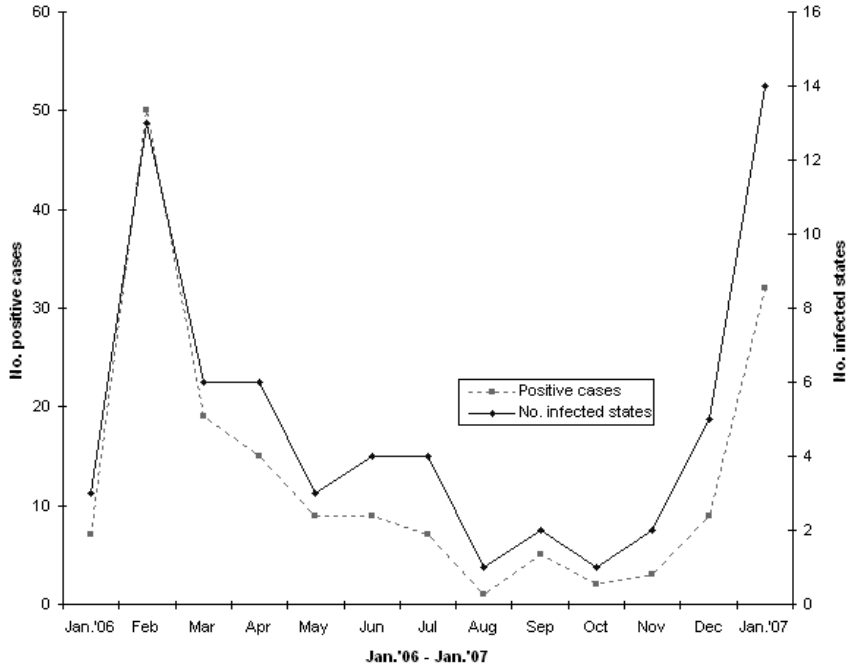

Figure2. Number of HPAI Positive cases in Nigeria (January 2006 – January 2007)

The dramatic decrease in incidence of HPAI in March through August is related to the restrictions imposed on movement of live poultry, pre-emptive slaughter of infected birds within and around infected farms and heightened public sensitization on the need for strict bio-security measures on farms (*2*). The number of cases and infected states were at the lowest in August. The number of cases and infected states gradually increased from November, 2006 with the number of infected States increased well above the initial peak in February 2006.

DISCUSSION

Between January 2006 and January 2007, some 166 poultry outbreaks of HPAI were confirmed and well over 828,000 poultry including commercial layers, breeder stocks of chicken, turkeys, ostriches, broilers, ducks, geese, local chicken and guinea fowls, had died of the disease or were culled in 19 States and the FCT, Abuja. Thus, as the virus persists in the poultry population in Nigeria, what started in 3 States has been reported in 49 Local Government Areas originating from 19 States and FCT. The H5N1 subtype which is known to be occasionally pathogenic to human was identified in each of the outbreaks. The first and yet only human H5N1 case was however confirmed in January 2007 in Lagos State, south-western Nigeria (*4*), 12 months after the first outbreak of the virus in the poultry population in the country. This situation further raises public health concern about the disease and its presence in Nigeria. The efforts of the animal health services thus needs more support from within and outside the country to effectively and efficiently control and stamp out the disease in Nigeria.

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POSTER PRESENTATIONS

USE OF MACROPHAGES IN SPLEEN, LIVER AND BONE MARROW OF *TRYPANOSOMA CONGOLENSE (KAURA STRAIN)* INFECTED MICE FOR LABORATORY DIAGNOSIS

Ayoade, R.A.

Ibadan, MNIM

ABSTRACT

At the time of sacrifice of each of the three groups, mice infected for two weeks showed slight decreased mean body weight from 21.5±6.8 to 20.2±5.9($p<0.05$). This was probably due to the severity of infection in proportion to high parasiteamia level with the trypanosome parasite competing with the body cells for nutrients. However, increased body weight in 4 weeks infected mice, 22±6.9 to 24.5±5.3($p<0.02$) was probably due to lack of reduced nutrient demand as a result of absence or reduced *Trypanosoma congolense* parasite in the blood. The marked splenomegaly noticed could be as a result of increase cell population per unit area with concomitant decrease in interstitial space (Anosa and Kaneko, 1984), while there were no significant liver changes except for slight hepatomegaly, 9.2±1.99($p<0.001$) of about only twice the weight of that of control (4.4±0.5). After the acute phase of infection, it began to return to pre-infection size.

The acute phase of the infection was characterized by significant decrease in PCV, Hb concentration, thrombocyte count but increased lymphocyte, neutrophil, monocytes and eosinophil numbers in the heart blood, but this is untrue for the 4wk infected mice as the PCV was close to those of control mice,47.6±4.4 and 49±1.6 respectively showing no obvious sign of anaemia. This may imply compensated haemolytic anaemia similar to that reported in trypanotolerant deer mice infected with T. brucei (Anosa and Kaneko, 1983a). However, there was depression of leucocytes and thrombocytes at this stage, significantly below those of the two weeks infected mice. This was due to continued proliferation and activation of macrophages in the bone marrow, liver and spleen.

There was a more increased phagocytic activity in spleen and liver of group A mice infected for two weeks but significantly lower in 4 weeks infected mice except in the bone marrow where phagocytic activity persisted and increased at the end of 4 weeks infection. This corresponds to the variations in sizes of the macrophages examined for phagocytosis.

In conclusion, the results of this study demonstrate that the macrophage plays a very vital role in the events in the spleen, liver and bone marrow of *T. congolense* infected mice, particularly with respect to cytophagia and the control of haemopoiesis, thus could be used to establish a confirmatory diagnosis for the above named strain of *Trypanosoma congolense*, which is gradually becoming endemic in the Niger-Delta and South-Western parts of Nigeria.

INTRODUCTION

Trypanosomes are flagellated protozoan parasites that live in blood and body fluids of their hosts. The trypanosome infections of laboratory animals (rats and mice) are characterized by anaemia, which are often severe (Mackenzie and Cruickshank, 1973; Anosa *et al.,* 1977; Anosa and Kaneko, 1983).

Due to their presence in blood, they produce numerous changes in its cellular and biochemical constituents (Anosa, 1988). Similarity of the onset of appearances and composition of the cellular infiltration into the liver to the cellular responses observed in the spleen of *T.congolense* infected mice probably reflects the fact that both organs are directly accessible to trypanosomes in circulation (Morrison *et al*., 1982), with preferential adhesion of trypanosomes to red blood cells in blood vessels in various body parts including the spleen and the liver (Anosa and Kaneko, 1983).

Macrophages constitute a significant proportion of inflammatory cells in spleen, liver and bone marrow with reported consequences of macrophage activation being two-fold (Mackenzie *et al*.,1973; Anosa and Isoun,1983; Anosa and Kaneko,1983a,b; 1989; Anosa et al.,1992).This is followed by phagocytosis of inflammatory cells and damaged resident cells in tissue (Anosa *et al,*1992) as well as induction of immunosuppression (Corsini *et al.,*1977).

The aim of this work is to compare the haematological changes, body, splenic and liver weight changes, as well as change in macrophage numbers, size and phagocytic function produced by *Trypanosoma congolense* infection in spleen, liver and bone marrow with a view to highlighting the differences and similarities observed with 2 weeks and 4 weeks infected mice. An attempt will be made also to compare the findings in the infected group with a control group so as to be able to establish a confirmatory diagnosis of the trypanosome parasite infection.

MATERIALS AND METHODS

Experimental animals

22 albino mice aged 6 – 8 weeks were used for this study. These mice were reared at the Experimental Unit, Veterinary Physiology Laboratory, and University of Ibadan, Nigeria. They consisted of 15 males and 7 females and were stabilized with oral tetracycline for 5 days before the commencement of the infection. The mice were divided into 2 groups, the infected group and control group. The infected groups were randomly subdivided into two. Group A included 5 males and 2 females, which were the controls, Group B included 5 males and 3 females infected for 2 weeks and Group C were 5 males and 2 females infected for 4 weeks. Only 5 mice of each of these groups were sacrificed at 2 weeks and 4 weeks respectively. They were fed compounded pellet feed and kept in cages with wood shavings throughout the period of the experiment.

Trypanosome

The Kaura strain of *Trypanosoma congolense* used for this study was obtained from National Institute of Trypanosome Research (NITR), Jos, Plateau State, Nigeria. This strain was first isolated from cattle in Kaura, Kaduna State, Nigeria in 1995. It has since June 2001 been maintained as an isolate passage in rats and mice.

The mice were infected by inoculating about 2.5 million parasites intraperiotoneally. This produced an initial acute infection with death of two mice on day 9 in Group A and 3 mice on day 11 in Group B.

Haematological techniques

The mice were bled intracardially after rendering them unconscious with ether. The control group (Group A) mice were also sacrificed at the end of 4 weeks. The blood was collected into vacutainer tubes containing the disodium salt of ethylene diamine tetra-acetate (EDTA). The PCV was determined by the microhaematocrit method. The erythrocyte (RBC), total leucocyte (WBC) and platelets counts were determined using a Neubaer haemocytometer, multiplying the values counted by 10,000µl, 50µl and 1,000µl respectively (E-MIL GOLD LINE BS748, United Kingdom).

Haemoglobin concentration (Hb) was measured with a haemoglobinometer (Coulter Electronics).

The erythocyte indices, mean corpuscular volume (MCV), mean corpuscular haemoglobin (MCH) and mean corpuscular haemoglobin concentration, were calculated using standard formulae (Jain, 1986).

This blood smears were stained with Giemsa's stain, these were used to evaluate changes in the peripheral blood cells and for differential leucocyte counts which were based on 100 cells per slide. Also reticulocyte count was based on 200 red blood cells per slide.

Collection of spleen, liver and bone marrow samples

After the mice were euthanized, the carcasses were opened up from the perineal region to the thoracic region. With the aid of a smooth tooth thumb forceps and a pair of scissors the spleen, liver, and kidney were dissected free and weighed with a meter balance (Mettler PM 600, Switzerland).

Impression smears of the spleen and liver of each mouse were made on two glass slides each and air dried. The femur of each mouse was dissected free; the head of each femur crushed and marrow contents milked out on a glass slide and immediately, a thin impression smear was made by sliding another glass slide in opposite direction to the one with marrow contents.

After air drying the impression smears from all the tissue samples, they were fixed for 3 minutes with methanol, air-dried again and then stained with Giemsa stain for 30 minutes after which the stains were then washed off.

Light microscopic examination of spleen, liver, and bone marrow smears

The phagocytic activities of macrophages in these smears were studied, Counting 50 macrophages from randomly selected fields of each smear. The differential and total number of cells engulfed by each macrophage was carried out using X 100 oil immersion Objective lens.

Statistical analysis was made by the Student's t test (Snedecor and Cochran, 1980). Data are given as mean ± standard deviation (SD).

RESULTS

Table 1. Mean body and organ weights of control and *T.congolense* infected mice

Mice Group	BODY WEIGHT		ORGAN WEIGHTS		
	Before Infection	At Sacrifice	Spleen (% body weight)	Liver (% body weight)	Kidneys (% body weight)
Controls(A)	19.4 ± 1.1	20.5 ± 2.5	0.4 ± 0.04	4.4 ± 0.5	1.3 ± 0.09
Infected(2 weeks)	21.5±6.8	20.2 ± 5.9***	3.4 ± 1.0*	9.2 ± 1.99*	1.9 ± 1.1***
Infected(4 weeks)	22 ± 6.9	24.5 ± 5.3**	1.7 ± 1.0*	5.1 ± 0.7**	1.3 ± 0.2#

* > Significant at P<0.001; ** > Significant at P<0.02; *** > Significant at P<0.05; # > Not significant at P>0.05

Table 2. Mean Erythrocyte Parameters and Thrombocyte counts of control and *T. congolense* infected mice

Mice Group	PCV (%)	Hb (%)	Reti-culocytes	MCH (pg)	MCHC (g/dl)	($X10^3\mu l$) Platelets
Controls(A)	49 ± 1.6	16.1 ± 0.4	0.6±0.9	28.1 ± 2.3	32.8 ± 0.4	173.6 ± 12.1
Infected(2 weeks)	33.4 ± 8.7**	10.8 ± 2.8*	8 ± 1.58**	17.5 ±2.2*	32.4 ± 0.3#	141 ±34.7**
Infected(4 weeks)	47.6 ± 4.4**	14.8 ± 1.2**	11 ± 3.4***	18.6 ± 0.7***	31.2 ± 0.7#	111.8 ± 9.3*

* > Significant at P<0.001; ** > Significant at P<0.02; *** > Significant at P<0.05; # > Not significant at P>0.05

Table 3. Mean absolute leucocyte differential values of control and *T.congolense* infected mice

Mice Group	WBC ($X10^3\mu$)	Lymphocytes	Monocytes	Segmented Neutrophils	Band Neutrophils	Eosinophils	Basophils
Control(A)	5.9 ± 0.9	4725 ± 606.8	13.5 ± 30.2	967.5 ± 182.4	99.3 ± 77.6	61.2 ± 60.55	13.5 ± 30.2
Infected (2 weeks)	8.4 ± 3.3*	7375.3 ± 3273.5*	189.2 ± 98.6*	1273± 1682.7*	119.6 ± 119.2**	157.2 ± 154.0*	–
Infected (4 weeks)	6.4 ± 1.5**	5640.5 ± 1250.1*	87.6 ± 27.4*	610.2 ± 370.4*	122.3 ± 119.5**	35.9 ± 33.5***	–

* > Significant at P<0.001; ** > Significant at P<0.02; *** > Significant at P<0.05; # > Not significant at P>0.05

Table 4. Mean number of cells phagocytosed by macrophages in bone marrow of control and T.congolense infected mice

Mice Group	No of MØ counted	RBC	Erythro-blasts	Reticu-locytes	Normo-blasts	Lympho-cytes	Neutro-phils	Baso-phils	Mitotic cells	Total No. of cells Engulfed
Control (A)	50	35.4 ± 5.7	0.2 ± 0.4	–	0.2 ± 0.4	–	0.4 ± 0.9	–	–	36.2 ± 4.7
Infected (2 weeks)	50	82.8 ± 8.5**	0.4 ± 0.9**	0.6 ± 0.9#	0.6 ± 0.5*	–	–	–	–	84.4 ± 7.9**
Infected (4 weeks)	50	103.2 ± 7.4*	2.2 ± 1.8*	0.4 ± 0.5*	2.4 ± 1.5*	0.2 ± 0.4*	1.8 ± 1.5**	0.2 ± 0.4**	–	110 ± 6.4*

Table 5. Mean number of cells phagocytosed by macrophages in spleen of control and T. congolense infected mice

Mice Group	No of Macrophage counted	RBC	Erythro-blasts	Reticu-locytes	Normob-lasts	Lympho-cytes	Neutro-phils	Baso-phils	Mitotic cells	Total No. of cells Engulfed
Control (A)	50	40.4 ± 5.5	–	–	0.6 ± 0.9	0.4 ± 0.9	0.4 ± 0.9	–	–	41.8 ± 5.6
Infected (2 weeks)	50	134.4 ± 20.9**	2.4± 1.1*	0.2 ± 0.4**	2.0 ± 1.6**	–	–	–	–	139 ± 22.6*
Infected (4 weeks)	50	84.6 ± 11.1*	1.0 ± 1.4**	–	2.2 ± 3.2*	1.4 ± 1.7**	–	–	-	89.3 ± 11.3*

* > Significant at P<0.001; ** > Significant at P<0.02; *** > Significant at P<0.05; # > Not significant at P>0.05

Table 6. Mean number of cells phagocytosed by Kupffer cells in liver of control and T. congolense infected mice

Mice Group	No of MØ counted	RBC	Erythro-blasts	Reticu-locytes	Normob-lasts	Lympho-cytes	Neutro-phils	Baso-phils	Mitotic cells	Total No. of cells Engulfed
Control (A)	50	34.8 ± 5.8	–	–	0.6 ± 0.9	–	0.2 ± 0.4	–	–	35.6 ± 5.2
Infected(2 weeks)	50	85.4 ± 26.5*	0.8 ± 1.1**	–	10.4 ± 23.2**	2.2 ± 3.0**	–	2.0 ± 3.5*	0.6 ± 0.9*	103.2 ± 13.5*
Infected(4 weeks)	50	68.4 ± 17.3*	4.4 ± 3.8**	–	5.0 ± 9.5**	0.8 ± 1.3*	3.0 ± 5.1*	3.8 ± 8.5**	–	85.4 ± 13.2*

* > Significant at P<0.001; ** > Significant at P<0.02; *** > Significant at P<0.05; # > Not significant at P>0.05

DISCUSSION AND CONCLUSIONS

The acute phase of the infection (2weeks infected mice) was characterized by significant decrease in PCV, Hb concentration, thrombocyte count but increased lymphocyte, neutrophil, monocytes and eosinophil numbers in the heart blood. In fact, this group of mice showed the highest and marked proliferation of granulocyte elements and proliferation and activation of macrophages with destruction of mature and maturing erythroids and granulocytic cells in the spleen and liver and to a lesser extent in the bone marrow at this phase of infection.

The chronic phase (4 weeks infected mice) did not show any obvious signs of anaemia but instead the PCV was close to the PCV of those of the control mice. This may imply compensated haemolytic anaemia similar to that reported in trypanotolerant deer mice infected with *T. brucei* (Anosa and Kaneko, 1983a). However, there was depression of leucocytes and thrombocytes at this stage, significantly below those of the two weeks infected mice. With continued proliferation and activation of macrophages (phagocytic activity) in the bone marrow and in the spleen and liver. There was phagocytosis of cells similar to those seen in the acute phase, erythroid hyperplasia, marked erythrocytes hyperplasia and return of the lymphocyte numbers (percentage) towards normal as shown by 5640 S± 1250.1 in 4 weeks infected mice and 4725 ± 606.8 in

control mice. This suggests that there is continued macrophage activation in the bone marrow with increased phagocytic activity as the infection progresses even when the phagocytic activity of the spleen and liver had decreased. This phenomenon in the bone marrow presumably contributes to progressive decrease in granulocytes and its precursors, thrombocytes and also erythroid series in the blood, leading to panleucopaenia. The changes explains why trypanosome infected animals and man succumb readily to secondary bacterial and other infections.

At the time of sacrifice of each of the three groups, mice infected for two weeks showed slight decreased mean body weight. This was probably due to the severity of infection in proportion to high parasiteamia level (acute phase of infection), with the trypanosome parasite competing with the body cells for nutrients. However, increased body weight in 4 weeks infected mice was probably due to lack of reduced nutrient demand as a result of absence or reduced *Trypanosoma congolense* parasite in the blood.

The splenic lesion in *T. congolense* infection in mice consisted of marked enlargement which could be as a result of a tremendous increase cell population per unit area with concomitant decrease in interstitial space and an obvious sludging of blood cells, particularly erythrocytes in many sinuses (Anosa and Kaneko, 1984) This increase in size and cell density could lead to splenic rupture which was the cause of death of two of the infected mice on day 9 post infection in this study. Another consequence of splenic enlargement and increase cell density is hypersplenism. There were no significant liver changes except for slight hepatomegaly, which after acute phase of infection begin to return to pre-infection size. The same trend was noticed for the spleen as well. There was marked bone marrow hyperplasia due to increased erythropoiesis.

A significant change in the spleen was paradoxical coexistence of splenic erythropoiesis and erythroclasis evidence as markedly increased numbers of normoblasts, reticulocytes and metarubricytes (late normoblast) on spleen impression smears. This agrees with the findings in mice infected with *T. brucei* (Anosa and Kaneko, 1983). The splenic macrophages were activated as indicated by their increased size and significant increase in numbers. There was marked increase in number of lymphocytes. Lymphocytes while decreasing in relative percentage increased overall due to the marked enlargement of the organ.

Splenic activities in *T. conglense* infected mice were major defensive with phagocytosis of parasites production of antibodies and cellular immunity involving transformed lymphocytes and macrophages, destruction of blood cells including erthrocytes, and to a lesser extent, neutrophils, thrombocytes, esinophils and erythropoiesis at tremendously increased rates (Anosa and Kaneko 1984).

The liver had no severe changes in *T. congolense* infection. There was proliferation and activation of Kupffer cells, (though scanty on the liver impression smears) with erythrophagocytosis as well as perivascular accumulation of lymphocytes. These two changes are related to the increased break down of red blood cells and intense antigenic stimulation respectively. Similar lesions have been found with light microscopy in deer mice infected with *T. equiperdum* (Moulton *et al.*, 1974), in *T. brucei* infected CFLP mice (Anosa *et al.*, 1977) and in

T. vivax infection of sheep and goats (Anosa, 1977). The cytophagia by macrophage in the bone marrow, first described in the bone marrow infected with *T. vivax* (Anosa *et al.,* 1992), definitely plays a major role in precipitating the pancytopeania and ineffective haemopoiesis in *T. congolense* infection. The process was selective for cell type and maturity with more matured cells of the erythroid and granulocytic series being preferentially phagocytosed, whereas lymphoid cell lies were seldom engulfed. This agrees with the fact that a macrophage simultaneously engulfed several apparently morphologically normal cell from different lineages suggest that the process is probably receptor mediated, presumably some receptors occurring in

some cell lines such as erythroid and granulocytic but not in lymphoid cells, and which are expressed as cells mature, are involved in producing the selectivity observed (Anosa *et al.,* 1997). Some non-cell-specific entity such as trypanosome antigen-antibody complex, which develops in trypanosomiasis and coats the target cells links the receptors on the target cells to other receptors on the macrophage leading to target cell macrophage adhesion and phagocytosis (Anosa *et al*., 1997)

There was a more increased phagocytic activity in spleen and liver of group A mice infected for two weeks but significantly lower in 4 weeks infected mice except in the bone marrow where phagocytic activity persist and increased at the end of 4 weeks infection. This corresponds to the variations in sizes of the macrophages examined for phagocytosis.

In conclusion, the results of this study demonstrate that the macrophage plays a very vital role in the events in the spleen, liver and bone marrow of *T. congolense* infected mice, particularly with respect to cytophagia and the control of haemopoiesis. Also, the results obtained could be used as a prototype for confirmatory laboratory diagnosis of the Kaura Strain of the *Trypanosoma congolense infection* which is rapidly spreading across various parts of Nigeria.

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My sincere gratitude goes to the Almighty God. I acknowledge the wonderful assistance of my supervisor, Prof. V.O Anosa.
I wish to dedicate this work to my loving aunt, Mrs Oluwanisola Aibor, may her gentle soul rest in perfect peace.

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MICROBIOLOGICAL AND ENVIRONMENTAL HYGIENIC EXAMINATIONS TO SELECTED BACTERIAL PATHOGENS IN DOG DROPPINGS

Egeler, S., Philipp, W. and Böhm, R.

Institute of Environmental and Animal Hygiene, University of Hohenheim, Stuttgart-Germany

SUMMARY

Due to the parasite *Neospora caninum* it was examined which other pathogens can be found in dog droppings and how their concentrations change over different periods of time.

The results of the microbiological examinations showed that the concentrations did not decrease much, so that dog droppings on pastures turn out to be a risk for livestock or humans.

The dog droppings were photographed weekly to document their decay, which took without protection about one-and-a-half month and with a shelter up to four months.

For the environmental hygienic examination the actual number of dog droppings on pastures was counted. The closer to towns the more dog droppings could be found.

Keywords: dog droppings, tenacity of pathogens, soil samples, actual number of dog droppings on pastures

1. INTRODUCTION

Currently there are about 5 million dogs in Germany, which results in about 1.500 tonnes of dog droppings each day (OHR and ZEDDIES, 2006).

This is not only an aesthetic but also a hygienic problem. A big discussion was initiated over a parasite called *Neospora caninum*, which causes aborts in cattle (SCHARES et al., 2005).

But there are several pathogens that can be found in dog droppings.

2. OBJECTIVES

In this study dog droppings were microbiologically examined for the presence and the concentrations of *Campylobacter* spp., *Clostridium perfringens*, *Enterococcus* spp., *Escherichia coli*, *Salmonella* spp. and the total number of bacteria. Furthermore pastures and meadows were checked for the actual number of dog droppings, because no any data was available.

3. MATERIAL AND METHODS

For the microbiological examination dog droppings were collected in animal shelters, numbered and set out on a meadow. The examination included two series.

There were 45 dog droppings in the first run of which 14 where examined microbiologically. In addition to the 14 dog droppings, droppings from a cow, a pig and a horse were examined for comparison.

Due to the rainy weather and the weekly sample drawing the first run was over after about one-and-a-half months, so that meaningful results could not be expected.

For this reason a second run was done, this time with a total of 39 dog droppings. Twenty of these droppings were put under a shelter to protect them against rain and direct sunlight so that a part of the samples could be saved for a longer time.

Ten droppings from under the shelter and ten without protection were microbiologically examined.

This run lasted about four months and samples were taken five times.

The dog droppings on the testgrounds of both series were photographed each week to document their decay.

During these two series soil samples were taken three times to assess if any bacteria in the droppings were washed out into the soil. The samples were taken in a depth of 1–3 cm from different points of the testground, a few from spots were dog droppings have been, others at the margin of the testground where no droppings have been.

The environment hygienic examination composed the stepping out of agricultural used greenland to acquire the actual number of dog droppings, because most of the studies to similar topics use the "worst-case-scenario", which is far away from reality. The meadows which were examined had different distances to residential estates.

Particularly suitable were meadows with a length of 200 metres and a width of about 100 metres. The meadows were divided in 0–50 cm, 0.5–2m, 2–5m and 5–10 m from the border.

4. RESULTS

4.1 Results of the microbiological examination

Pathogen	First run	Second run	Soil samples
***Campylobacter* spp.**	Found in the cow (50% *C. coli,* 50% *C. jejune*) and in the pig (92.6% *C. fetes fetes*) droppings	Found in one dog dropping, clear detection under the microscope	Not found
Clostridium perfringens	Concentrations ranged between 10^2 und 10^7. Concentrations of horse and cow samples very low (about 10^1), later no more detectable.	Concentrations ranged between 10^3 and 10^7	Concentrations under elapsed droppings ranged between 10^4 and 10^5, rest ranged between 10^2 and 10^3
***Enterococcus* spp.**	Concentrations ranged between 10^3 and 10^9	Concentrations ranged between 10^4 and 10^9	Concentrations under elapsed droppings were around 10^4, rest were about 10^3
Escherichia coli	Concentrations ranged between 10^4 and 10^7	Concentrations ranged between 10^3 and 10^9	Concentrations under elapsed droppings were around 10^5, rest were about 10^2
***Salmonella* spp.**	Not found	Found in two dog droppings, *Salmonella* spp. belonged to the group Anti O–4.5.	Found under one of the elapsed dog dropping which contained *Salmonella* spp., also Anti O–4.5
Number of whole bacteria	Concentrations ranged between 10^6 and 10^{10}	Concentrations ranged between 10^7 and 10^{10}	Concentrations ranged between 10^7 and 10^8

Campylobacter spp. was detected only in the fresh dropping samples and could not be detected later.

The concentrations of *Clostridium perfringens* in the horse and the cow dropping samples were very low. The reason may be the exclusively vegetarian alimentation.

The concentrations of the examined pathogens did not decrease as expected what may be due to the different spots on the droppings from where the samples were taken.

The results of the soil samples indicate a possible washing out of some of the bacteria to the soil during the decay.

4.2 Results of the environmental hygienic examination

The results of the environmental hygienic examination were as expected: a lot of dog droppings were found close to towns and on the borders of pastures and meadows.

It was conspicuous that in the areas where a green corridor exists on the opposite side of the walk, many more of the dog droppings were found there (up to 19 droppings on 200 metres).

The larger the distance to towns the fewer dog droppings were found, sometimes no dog dropping was found.

The number of the dog droppings depends strongly on the walk. There are some walks that are highly frequented by dogs; others close to towns seem not as attractive for walking dogs.

The most contaminated meadow was located near to highly frequented roads and not too far away from town, so it can be reached easily by foot or by car. On this meadow 42 dog droppings were found within the first ten metres from the border.

The total decay of the droppings without protection took an average of one month, with the protection of the shelter up to four months. The shelter surely does not reflect the real conditions but it is close to the circumstances if the dropping is for examples set under a tree or a bush.

A big influence on the decay of the droppings had the weather but also insects like worms, beetles or flies contribute to the decay, because some of them need the droppings as nutrition.

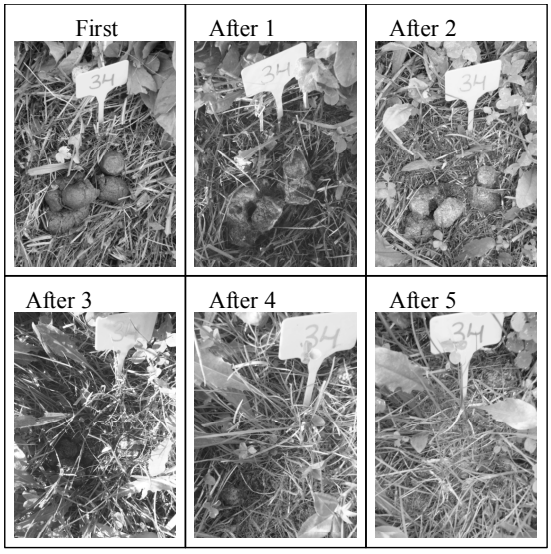

Figure 1. Example for the decay of a dog dropping without protection

5. CONCLUSIONS

The results of the laboratory examinations show that each dog dropping on meadows and pasture is a potential and long-term disease-source for livestock or humans.

Thereby not only grass for feeding is endangered, but also herbal products like crops or fruits

So pathogens in dog droppings can enter the food chain of humans by contaminated meat, crop or fruit.

Solutions to reduce the contamination of the environment with dog droppings can be the installation of so called “dropping stations” on strongly frequented walks where the dog owners find plastic bags and bins for disposal of their dog’s droppings, or special “dog meadows” in or close to residential areas, which are cleaned up regularly by the sanitation department or by voluntary dog owners.

Additional informative signs should be placed in highly frequented areas to inform the population about possible risks that can come from dog droppings.

Some farmers provide marked border areas of their pastures to dogs, which is a real good possibility to keep the dogs away from the grass that is used for feed.

Another alternative may be the enclosure of the agriculturally used areas, but that goes along with high costs and endeavours for the farmers.

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SEROLOGICAL SURVEY OF ANTIBODIES AGAINST *TOXOPLASMA GONDII* IN ORGANIC SHEEP AND GOAT FARMS IN GREECE

Ntafis, V.[1], Xylouri, E.[1], Diakou, A.[2], Sotirakoglou, K.[3], Kritikos, I.[1], Georgakilas, E.[1] and Menegatos, I.[1]

[1] Department of Anatomy & Physiology of Farm animals, Faculty of Animal Science, Agricultural University of Athens, 75, Iera Odos Str., 118 55, Athens, Greece; [2] Laboratory of Parasitology and Parasitic diseases, Veterinary Faculty, Aristotle University of Thessaloniki, 54 124, Thessaloniki; [3] Department of Mathematics and Statistics, Faculty of Science, Agricultural University of Athens, Agricultural University of Athens, 75, Iera Odos Str., 118 55, Athens, Greece.

SUMMARY

In the present study, a preliminary and a main research for IgG antibodies against *Toxoplasma gondii* were conducted in organic sheep and goat farms of Western Greece, by means of ELISA. In the preliminary research, 413 sera were tested and the significant resulting seroprevalence triggered further research that was conducted one year later. For the main research, 349 sera were tested. Results indicated that seroprevalence against *Toxoplasma gondii* is high in organic farms, both in animals that had or had not aborted, is more prevalent in older animals and susceptibility alters between gender and species.

Keywords: *Toxoplasma gondii*, sheep, goats, organic, ELISA, Greece

INTRODUCTION

Toxoplasmosis is a parasitic zoonosis caused by the protozoon *Toxoplasma gondii*, an intracellular parasite which infects a wide range of animal species. Over two hundred of terrestrial and marine mammals (Haralambidis 1995, Dubey *et al*. 2003), along with birds (Dubey 2002) and human seem to be intermediate hosts of this parasite. Members of the family Felidae, such as domestic cat, act as definite hosts (Urquhart *et al*. 1996).

Sporulated oocysts, bradyzoites encysted in tissue and tachyzoites are the three infectious stages in the life cycle of *T. gondii*. These stages are infectious for both intermediate and definite hosts (Bowman 1999).

Herbivores are mainly infected by oral ingestion of sporulated oocysts (Jubb *et al*. 1993), which are very resistant to environmental conditions (Tenter *et al*. 2000). In intermediate hosts, such as sheep and goats, primary exposure to *T. gondii* during gestation can lead to vertical transmission of toxoplasmosis that occur transplacentally to the fetus (Tenter *et al*. 2000) and it is believed that may occur more than once and in successive litters (Buxton *et al*. 2006, Duncanson *et al*. 2001). In several species, such as sheep, goats and cattle, *T. gondii* transmission occurs after consumption of tachyzoites' contaminated milk (Powell *et al*. 2001). In sheep, venereal transmission is also probable (Martinez-Garcia *et al*. 1996). In all cases, *T. gondii* forms tissue cysts after host infection (Bowman 1999).

Toxoplasmosis in sheep is mainly asymptomatic, except for the occasional fever and tachypnea during the first days post infection (Esteban-Redondo *et al*. 1999). Goats however and especially kids can develop anorexia, diarrhoea, dyspnoea and even death when they are infected with oocysts (Dubey 1989). Fetal deaths, mummifications, abortions and birth of stillborn or weak lambs and kids are the most frequent symptoms in flocks of sheep and goats, being infected with *T. gondii*. Abortions are more frequent after mid-gestation (Esteban-Redondo and Innes 1997).

Till today toxoplasmosis research carried out in Greece, referred only to conventional sheep and goat farms (Haralabidis 1987, Stefanakis *et al*. 1995, Kontos *et al*. 2001, Diakou *et al*. 2005a, Diakou *et al*. 2005b) and no data are available for the organic farming, which is a strongly developed sector.

The aim of the present study was to detect antibodies against *T. gondii* in organic flocks of sheep and goats at Western Greece and to evaluate the parasite's potential role in the manifested abortions.

MATERIALS AND METHODS

Sampling

The present study was conducted in two phases: preliminary and main. For the preliminary research, in 2005, blood samples were collected from 413 animals (47 ewes and 116 goats that aborted during the reproductive period of 2004–2005, 137 rams and 113 male goats) from 11 sheep, 8 goat and 8 mixed organic flocks of Peloponnese and Western Sterea Hellas and antibodies against *T. gondii* were detected.

For the main research, in 2006, blood samples were collected from 349 animals (23 ewes and 49 goats that aborted during the reproductive period of 2005–2006, 107 ewes and 85 goats that did not abort, 52 rams and 33 male goats) from 7 sheep, 4 goat and 2 mixed organic flocks of Peloponnese, Western Sterea Hellas and Ioannina County. All samples were categorized into three groups: adult males, females that aborted during the reproductive period 2005–2006 and females that did not abort during the same reproductive period. The first two groups are consisted of relatively few animals while the rest animal population is the largest. For this reason, the sample size per flock was chosen to represent 25% of total males and females that aborted and 7% of females that did not abort.

Blood was collected from the jugular vein using vacutainer tubes without anticoagulant. Blood samples were placed in cryo-boxes and were transported to the lab within 8 hours. Serum was collected after centrifugation (2000 *rpm* for 20 minutes) in 1,5ml vials and kept frozen at –20ºC until tested.

Antibodies detection

T. gondii specific IgG antibodies were detected by means of enzyme-linked immunosorbent assay (ELISA). The assay was completed in four stages (Haralabidis 1984). Antigen (*in vivo* cultured parasites) was diluted in a buffer solution ($NaHCO_3$–Na_2CO_3, pH 9.6) and it was added in flat bottom, polystyrene microtiter plate of 96 wells. After a two hour incubation in room temperature and washing of the microtiter plate with distilled water containing 0.05% Tween-20, animal sera diluted 1:300 in PBS, pH 7.2, were added in the microtiter plate. Three pairs of control sera in each plate were used. After incubating and washing the microtiter plate as before, during the third stage, the conjugate was added: Anti-Sheep IgG, whole molecule Sigma A-5187 (diluted with

PBS, pH 7.2) was used for sheep sera and Anti-Goat IgG, whole molecule Sigma A-4187 (diluted with PBS, pH 7.2) was used for goat sera. During the fourth stage, after incubation and washing of the microtiter plate, enzyme substrate (p-Nitrophenyl Phosphate, Sigma 104 Phosphate Substrate), diluted in buffer solution ($NaHCO_3$–Na_2CO_3, pH 9.8) was added. After a ten minute incubation, stop solution, 0.1–3N NaOH was placed in every microtiter plate well and test results were being analyzed using a photometer (HUMANREADER, HUMAN Diagnostic Systems, Germany) at 405 nm wave length.

Results were estimated after the determination of the cut off value. Cut off value was determined by adding to the average of the optical density (OD) of negative control sera values their threefold standard deviation:

$$\text{Cut off} = \text{Ave} + 3\text{SD}$$

Serum samples with OD values, higher than the cut off were considered to be positive, whereas serum samples with OD values lower than or equal to the cut off were considered to be negative (Haralabidis 1987).

Statistical Analysis

Statgraphics Plus 4.0 statistical package was used in the comparison of proportions. P-values<0.05 were considered significant at 5% confidence level.

RESULTS

In the preliminary research, in sheep, 63 (45.99%) out of 137 rams and 33 (70.21%) out of 47 ewes that aborted were positive and in goats 22 (19.47%) out of 113 male goats and 29 (25%) out of 116 female goats were positive.

In the main research, in sheep, 20 (38.46%) out of 52 rams, 14 (60.87%) out of 23 ewes that aborted and 58 (54.21%) out of 107 ewes that did not abort were positive. In goats, 4 (12.12%) out of 33 male goats, 7 (14.29%) out of 49 goats that aborted and 19 (22.35%) out of 85 that did not abort were positive.

In sheep, rams had the lowest seroprevalence and ewes that aborted the highest. Ewes of both groups had significantly higher seroprevalence than the rams ($p<0.05$). The difference in seroprevalence between the two female groups was not statistically significant ($p>0.05$).

In goats, the lowest seroprevalence was that of the males, though there was no statistically significant difference between the three groups ($p>0.05$). Seroprevalence in sampled goats was significantly lower than that in sheep in all three groups ($p<0.05$).

In addition, based on age criteria, sheep and goats were categorized in two age groups of 0–4 years old and older than 4 years. In sheep, seroprevalence for animals of 0–4 years old was 39.85% and for animals older than 4 years was 61.97%. In goats, seroprevalence for the same age groups was 13.04% and 24% respectively. Seroprevalence in both species increased significantly with age ($p<0.05$).

DISCUSSION

In Greece, organic sheep and goat farming is of major economic importance and it possesses the largest sharing portion of all farmed animals (79% of the total, with 49% attributed to goats and 30% to sheep) (Ministry of Rural Development and Food, 2004). Sheep and goats get infected with *T.gondii* mostly with oocysts that are excreted in the environment via cat faeces (Urquhart et *al*. 1996). Cats are mainly used for the elimination of rodents and have access to animal storage rooms where feedstuffs can get contaminated by their faeces. In some cases, infected cats do not stay in the farm but are located in the overall area and they have occasional access to feedstuff storage rooms.

The preliminary research revealed for the first time that in organic sheep and goat farms of Greece (Peloponnese and Western Sterea Hellas) a significant number of animals are infected with *T. gondii*, a zooanthroponotic pathogen. It was surprising that animals tested one year after they had aborted, still had a high antibody titre indicating the strong immune response to the parasite. Difference in seroprevalence observed between sheep and goats and between males and females (Innes 1997, Roberts *et al*. 2001), further triggered us for the main research.

The main research revealed that the comparison of the proportions of the seropositive female animals that had abortions during reproductive period 2005–2006 and the female that did not have abortions during the same reproductive period did not have significant differences both in sheep and goats. The disease seems to be enzootic among farms and for this reason it is not expressed with massive abortions. Evaluating the relation between animal sex and difference in susceptibility against *T. gondii,* males have lower proportion of seropositive animals compared to the results of the female animals in both species. Between the two different species tested, the results are significant only in sheep. Female animals are more susceptible compared to males, because of their hormone profile (Roberts *et al*. 2001). This is due to the effect of estradiol and progesterone, on non specific (Walker *et al*. 1997) and specific immunity against *T. gondii* (Roberts *et al*. 1995).

The main research also suggests that goats have significantly lower proportion of seropositive animals in all three groups compared to sheep. Those results are in agreement with those mentioned in similar surveys (Tenter *et al*. 2000, Van der Puije *et al*. 2000) and can be explained by the difference in susceptibility among the intermediate hosts of *T. gondii.* Some scientists believe that different species susceptibility is related to how quickly the immune system can produce the key cytokine IFNγ and mention that goats are more resistant than sheep concerning infection of the parasite (Innes 1997). Goats that are reared in those organic farms seem to be more susceptible than sheep. However, some other researchers demonstrate that goats have more severe clinical symptoms of toxoplasmosis that can be result to death (Dubey 1989, Kaufmann 1996). If the above statement is correct, the higher mortality of goats compared to sheep may keep the proportion of seropositive animals at lower levels.

The proportion of *T. gondii* seropositive animals increases with age in both sheep and goats. This can be attributed to the fact age increases the possibility of infection. Taking into consideration that toxoplasmosis is a chronic disease (Esteban-Redondo and Innes 1997) and that infected sheep and goats are seropositive for a long lasting period (Conde *et al*. 2001) it is expected that older animals have higher proportions of seropositive individuals. It can also be noted that seroprevalence in those organic farms is high enough to conclude that the disease is enzootic. In all cases, special attention should be given to the storage of organic feedstuffs and management of organic pasture, in order to prevent their contamination by infected Felidae.

In conclusion, the results of the present research justify the presence of IgG antibodies against *T gondii* in organic sheep and goat farms in Western Greece. They also demonstrate that the disease in these farms is enzootic while there is different susceptibility between goat and sheep and between sexes. Further research is needed to clarify the proportion of sheep and goats that have antibodies against *T. gondii* in the total number of organic farms in Greece and also to reveal the different susceptibility among those species.

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POSSIBILITIES OF PARATYPHOSIS PREVENTION IN BROILERS

Ţibru, I., Orlič, D. and Cătană, N.

Veterinary Medicine Faculty of Timişoara 300645, Calea Aradului 119,
tibru_ioan@yahoo.com

ABSTRACT

Under intensive growing systems, broilers, due to their genetic way of reproduction, are exposed to many stress factors. All these reflect on the productive parameters (average daily gain, weight at slaughtering) and represent a problem for farmers. This category also includes the risk factors caused by immunosuppression and by pathogenic germs` direct action (*Salmonella* spp., coliform bacteria), which are the veterinary doctor`s task.

Successive to the removal of antibiotics from animal food, between 1980 and 1990, most antibiotics used as growth promoters were also removed from E.U. market, and the last one was removed in January 2006. So there have been many attempts of proposing and then of applying substitutes, which should assure a bigger production and a protection with at least the same level like that one provided by antibiotics.

Organic acids have been used successfully in pig growth for 25 years and they still remain an alternative solution. Although they have been less used in poultry rearing, they are still very efficient if they are adapted to poultry physiology.

Another pretender at antibiotic replacement is represented by essential oils, and that is why many experiments have been performed in order to get to know essential oils` way of application. Mass media and scientific works use various names, like: plant extracts, phytogen additives etc., the following classification being proposed:

- essential oils – the ones obtained from plants belonging to the volatile category with a specific smell and/or other properties, and these plants are used especially in perfume, flavour and pharmaceutical industry;
- herbs – flowered plants whose stem above land does not become woody, plants appreciated for their medical properties, taste, smell;
- medicinal plants – from which the active substance present in roots, leaves or bark could be extracted.

The use of acidifiers or other such products starting with broilers` first day of life is a practiced in all aviculture. They are administrated in order to prevent the development of pathogenic germs, to increase digestibility and to favour intake. In the last years, acidifiers have been used in order to protect broilers` digestive tract from *Salmonella* invasion.

MATERIALS AND METHODS

We have taken under study 15,000 broilers, Ross 308, distributed into three groups of 5,000. All broilers were taken from a *Salmonella*-free incubation station and then housed and fed according to the requirements of this hybrid. The experimental group (E_1) received through forage Multiacid

P, 2 kg/tone; the experimental group (E_2) received Multiacid P, 3 kg/tone, and the control group M received 0.5% amoxicillin during the first seven days.

Then each group was divided into two (A and B), and each lot was administrated a *Salmonella* spp. culture, $10x10^9$ UFC/chicken.

Along the experiment, we have supervised mortality, specific intake, average weight, bacteria presence through cloacal swabs and, in the end we have performed the bacteriological control upon liver surface and the craw and caeca pH.

The product Multiacid P comprises the following acids: propionic, formic, lactic and plant extracts such as eugenol, carvacol, extracted from *Origanum vulgare*.

RESULTS AND DISCUSSION

Multiacid P components manage to reduce the pH within the broilers` digestive tract, impeding *Salmonella* engraftment and development. Table 1 presents craw and caeca pH in the case of all three broiler groups, and also *Salmonella* presence or absence in the liver.

Table 1. pH values of digestive tract content and presence of *Salmonella enteritidis* in liver

	pH of crop content		pH of ceca content		Positive to *S. enteritidis*		
	Day 12	Day 22	Day 12	Day 22	Day 1	Day 12	Day 22
E 1	4.8	4.3	4.6	4.3	+	–	–
E 2	4.6	4.4	4.4	4.3	+	–	–
M	5.8	5.7	5.6	5.3	+	–	–

Considering the productive parameters presented in Table 1, we may observe the pH reduction as a result of the administration of Multiacid P, compared to the control group. We may also notice that, after 12 days, no *Salmonella* could be observed.

According to the productive parameters presented in Table 2, we may notice that the product is a good eubiotic one, allowing a good-natural bacterial flora which accomplishes an unspecific local immunity, impeding the development of pathogenic germs.

Table 2. pH values of digestive tract content and presence of *Salmonella enteritidis* in liver

	pH of crop content		pH of ceca content		Positive to *S. enteritidis*		
	Day 12	Day 22	Day 12	Day 22	Day 1	Day 12	Day 22
E 1	4.8	4.3	4.6	4.3	+	–	–
E 2	4.6	4.4	4.4	4.3	+	–	–
M	5.8	5.7	5.6	5.3	+	–	–

The analysis of the values in Table 2 permits us to support the idea that the application of Multiacid P has led to a reduced mortality, a smaller specific intake and a bigger average weight.

CONCLUSIONS

1. Acidifiers assure an anti-*Salmonella* protection in broilers, during growth period.

2. The mixture between organic acids and plant extracts and essential oils increases the degree of anti-*Salmonella* protection in broilers.
3. The product Multiacid P allows a good combination and permits the anti-*Salmonella* protection, the reduction of specific intake and a bigger body weight.

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STAPHYLOCOCCUS AUREUS MASTITIS INVESTIGATION ON HERD LEVEL ON A DAIRY FARM IN HUNGARY

Tirián, A.[1], Kovács, M.[1], Brydl, E.[2], Szenci, O.[2], Könyves, L.[2], Jurkovich, V.[2] and Tegzes, L.[2]

[1] University of Kaposvár, Kaposvár, Hungary; [2] Szent István University Fac. of Vet Sci, Budapest, Hungary; Corresponding author: tirian@tvn.hu

SUMMARY

A herd level investigation was performed on a dairy farm in Hungary to determine the incidence of *S. aureus* intramammary infection (IMI). Because of the expected high number of infected cattle a filtering method based on the SCC values was used to select cows for sampling. The filtering method showed partial success, the incidence of the infection in the selected population was significantly higher than the prevalence of the whole herd.

INTRODUCTION

Staphylococcus aureus mastitis is one of the most common diseases in the dairy production. The estimated losses in Hungary are close to 400 Euros per lactation year for each infected cow (1).

The *S. aureus* caused mastitis is a contagious disease. The infection can happen horizontally and vertically in the herd. In case of horizontal infection the cows are infected mostly during milking (4). By the vertical process is the feeding of contaminated milk and the fly invasion of the farms of capital importance (2, 3).

The elevated somatic cell count (SCC) is a sign of the infection. Since *S. aureus* IMI is very difficult to treat and the bacteriological cure rate is usually below 30% (5) the infected animals have despite treatment a constantly elevated SCC value or repeatedly high values throughout the lactation period.

OBJECTIVE

Goal of the study was to determine the incidence of Staphylococcus aureus intramammary infection (IMI) on a large scale dairy farm in Hungary using a preselection of the dairy cows based on the somatic cell count (SCC) values of the last 14 months.

MATERIALS AND METHODS

The study was conducted on a large scale dairy farm in Hungary with 595 milking cows. The bulk tank milk SCC was above 1.000.000 cell/ml, and previous *S. aureus* IMI's were determined, 40 infected cows were kept separately. The data of the previous 14 monthly milk controls were used to select the cows for milk sampling. As threshold values were 500.000, 1.000.000 and

2.000.000/ml chosen. All cows having SCC values at least three times or at two different IMI occasions (between two high SCC data at least two monthly record was below 400.000) have been selected for sampling.

As preevaluation for the three different threshold values were used the data of the previously known S: aureus cows. The sensitivity according to the preevaluation was the highest using 500.000 cell/ml as threshold, but the number of cows selected for sampling was close to the number of the whole herd (the population involved also some culled animals). In case of 1.000.000 as threshold value the preevaluation showed a high sensitivity and the size of the population was also acceptable, by using 2.000.000 the sensitivity was very low (Fig. 1)

Table 1. Preevaluation of the different threshold values

Treshold value	**Population sampled**	***S. aureus* pos. Population**
500.000	580	40 (100%)
1.000.000	388	36 (90%)
2.000.000	244	23 (57,5%)

According to the data of the preevaluation 1.000.000 has been chosen as threshold value. Altogether 209 cows have been selected for sampling (some cows of the selected population were dried off, were in the treated mastitis group or were culled in the previous 14 months) and 809 quarter milk samples were taken at the morning milking. The samples were cooled and transported to the laboratory of the Szent István University, Faculty of Veterinary Science, Department of Animal Hygiene. The samples were frozen for 5 days, then after thawing were streaked on Columbia sheep blood agar. For identification of the bacteria culturing on Baird-Parker agar and Staphylase test (Oxoid Diagnostic Reagents) was used. The antibiotic resistance was determined via plate diffusion test on Müller-Hinton agar plates (Fig 2).

RESULTS

Out of the sampled 209 cows 121 were S: aureus positive (58%). The number of known infected cows in the whole herd was 161 (26%).

According to the high incidence of *S. aureus* IMI an eradication protocol was implemented. The main points were following:

- All infected cows should be kept separated from the *S. aureus* negative cows. The positive cows should be milked at last in the milking house. After drying off the *S. aureus* positive cows can be kept together with the *S. aureus* negative population, but 3 weeks before the expected parturition should be separated from them.
- Infected cows should calve in a separated calving barn. The calves should receive colostrum of a *S. aureus* negative cow. The feeding of mastitis milk or milk of the high SCC group to the calves is prohibited.
- *S. aureus* positive cows with clinical mastitis should be treated according to the results of the antibiotic resistance test.
- Changes in the milking technology were suggested. Most important point of the changes was the disinfection of the milking units between two consecutive milkings to avoid spreading of the infection during milking.

– Sampling protocol should be following: All cows with clinical mastitis should be sampled. Cows with high SCC value should be tested with CMT, positive quarters should be sampled. Every freshly calved first parity cow should be sampled in the first 5 day after calving.

Table 2. Antibiotic resistance of the *S. aureus* bacteria

	2343	1988	2239	2170	1328
penicillin	+	+	+	+	+
streptomycin	+/–	+/–	+/–	+/–	+/–
ampicillin	+	+	+	+	+
cloxacillin	+	+	+	+	+
amoxi-clav	+	+	+	+	+
cefalexin	+	+	+	+	+
linkomycin	+	+	+	+	+
	1334	1926	2761	2034	1548
penicillin	+	+	+	+	+
streptomycin	+/–	+/–	+/–	+/–	+
ampicillin	+	+	+	+	+
cloxacillin	+	+	+	+	+
amoxi-clav	+	+	+	+	+
cefalexin	+	+	+	+	+
linkomycin	+	+	+	+	+

In the following months 449 samples were sent to our laboratory, 97 of them were *S. aureus* positive. Out of 75 first parity cows 18 (24%) freshened with *S. aureus* IMI after calving. Despite the high culling rate, the incidence of *S. aureus* infection on herd level is close to 30%.

CONCLUSIONS

The filtering method used in our study can be a useful tool in the selection of cows for *S. aureus* sampling, although there is a risk leaving infected animals unsampled. The high number of *S. aureus* positive samples after the filtered sampling can be due to left infected animals out of the sampled population but also due to new mastitis cases. The high prevalence of *S. aureus* IMI in the first parity cows shows the important role of feeding mastitis and high somatic cell milk to the calves.

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SALMONELLA RODS PREVALENCE IN WATERFOWL IN SOUTH-EASTERN POLAND OVER 2001–2005

Trawińska, B., Polonis, A., Saba, L. and Chmielowiec-Korzeniowska, A.

Department of Animal Hygiene and Environment, University of Agriculture, Lublin, Poland

ABSTRACT

The present work aimed to evaluate prevalence frequency of Salmonella rods as well as serovars isolated from goose and duck flocks investigated in the years 2001–2005 in the south – eastern part of Poland.

The work was performed on the grounds of the data supplied by the Regional Laboratory of Veterinary Hygiene in Lublin. The material for examination was made by the cloacal swab cultures and the internal organs (hearts and livers) taken from the flocks of geese and ducks, slaughter, layer and reproductive, maintained in the south-eastern part of Poland Salmonella rods were isolated inoculating into selective-multiplying media and differentiating according to the procedure applied currently. Salmonella was classified into serovars using the glass agglutination test with diagnostic sera.

Prevalence frequency of Salmonella rods in the south-eastern Poland in the years 2001–2005 in the goose flocks reached 5%,whereas in ducks – 6,8% at all the studied flocks. Total bacteria obtained from geese on the basis of cloacal swabs constituted 3,6% of the investigated flocks, whereas from the internal organs – 1,2%. The presence of *S. enteritidis* (44,4%) was recorded in goose flocks most frequently and S. typhimurium (33,3%) more rarely. In ducks, though, S. Enteritidis (27,3%) as well as S. Hadar and Salmonella from group B (ca 18%) and S. from group C, C_1, C_2, E was reported. The reproductive geese were free from infections developed by salmonella, while these bacteria were identified in the slaughter geese. The highest infection level in geese and ducks was detected in 2001, it was 8% in the goose flocks and 11% in the duck ones. In the years 2002 and 2005 no cases of ducks infected with salmonella were recorded.

The more stringent requirements concerning the standard zoohygienic conditions as well as obligatory monitoring of flock health state resulted in a decrease of Salmonella infections rate in waterfowl. Detectability of these organisms did not show any serious differences as compared to their presence in the waterfowl in Poland as well as in other EU states.

Keywords: *Salmonella* rods, geese, ducks

OBJECTIVE

The extensive commercial water fowl breeding comprising a high number birds poses a threat of various infectious diseases incidence, in that salmonellosis.

In the breeding farms, the water fowl is usually reared in the outdoor voliers that may increase birds` exposure to infection with salmonella frequently found in feedstuffs, livestock, wild animals, rodents and other free living birds. The microbes are also transmitted by the poultry

hatcheries as pathogens` presence was confirmed in 64% of samples obtained there [Hoszowski and Wasal, 2005].

Among the salmonella isolated from the geese, the following serovars were detected: *S. Typhimurium, S. Enteritidis, S. Anatum* and *S. Thompson* [Samorek-Salamonowicz et al., 1998]. The Salmonella rods are characterized by long survivability in the environment. It was shown that S. Typhimurium bacteria can survive in ducks droppings for about 190 days, while in the duck yard substrate even up to 240d [Szeleszczuk, 1998]. Despite the bird monitoring for Salmonella rods [Davies et al., 1997; Trawińska et al., 2003] as well as the pathogens control, they have been still hazardous for human and animal health.

Therefore, the objective of the present work was to assess the salmonella prevalence in duck and goose flocks in the south – eastern part of Poland over 2001–2005.

METHODS

The work was realized on the basis of the findings provided by the Regional Laboratory of Veterinary Hygiene in Lublin. Prevalence of Salmonella rods in geese and ducks was evaluated in the south – eastern region of Poland during the years 2001–2005.The studies also included bacteria classification into serological groups. The examination material was constituted mainly by the cloacal swab cultures collected from the layer, reproductive and slaughter geese as well as ducks. There were investigated diseased and died birds, in that day -old chicks. Salmonella rods isolated from the internal organs – hearts and livers, were inoculated on the selective-multiplying and differentiating media according to the procedure applied currently. Salmonella pathogens were classed into serovars on the grounds of the glass agglutination test with diagnostic sera.

RESULTS

Prevalence of Salmonella rods in the water fowl flocks is presented in Table 1.The examinations towards Salmonella – induced infections were carried out in 971 goose flocks and 218 duck flocks over 2001–2005.The studies covered 794 slaughter goose flocks (the highest rate) and 57 layers (the lowest rate). Throughout the research period, the reproductive geese did not show any signs of infection caused by these pathogens. As for geese layers, Salmonella presence was reported only in one flock, which made up 1,7% of total number. The highest rate of geese infected by Salmonella was recorded in slaughter goose flocks, where in 2001 this pathogen percentage proved the highest (8,1%) and the lowest (1,8%) in 2002. Prevalence frequency of Salmonella rods in geese reached 3,7% whereas in ducks – 5,0%. The studies run in the duck flocks in 2002 and 2005 did not reveal the presence of these bacteria in any flock. The highest infection level in the ducks was recorded in 2001 – ca 11%, while the lowest in 2004 (2,8%). Besides, salmonella classification into serologic types was performed and given in Table 2.It was shown that among the serovars determined in the goose flocks, S. Enteritidis prevailed (44,4%). Salmonella from this serological group were detected most frequently in 2001 (66,6% of infected flocks), while S. Typhimurium pathogens were reported more rarely (33,3%) in these birds. Moreover, S. Dublin, S. Derby and Salmonella from B and C group were also determined, yet to a smaller extent. In the duck flocks, no presence of *S. Typhimurum* was confirmed. However, *S. Enteritidis* pathogens (27,3%) as well as S. Hadar and from group B, C, C_1, C_2 and E were detected.

CONCLUSION

The highest percentage of Salmonella infections in both geese and ducks was reported in 2001. The more stringent requirements concerning the standard zoohygienic conditions as well as the obligatory monitoring of the flock health state decreased a Salmonella pathogens infection rate in the water fowl flocks in the successive years, especially in 2005. Salmonella detectability in the water fowl from south – eastern Poland, Salmonella Enteritidis, did not show any substantial differences as compared to their presence in Poland or other EU member states [Bugajak and Bugajak, 2002].

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Table 1. Salmonella rods presence in goose and duck flocks

Poultry	Flocks studied	Number of infected flocks	% infected flocks	Year
Layers geese	19	1	5,3	2001
	10	0	0	2002
	7	0	0	2003
	13	0	0	2004
	8	0	0	2005
Total	57	1	1,7	2001 –2005
Reproductive geese	20	0	0	2001
	25	0	0	2002
	36	0	0	2003
	18	0	0	2004
	21	0	0	2005
Total	120	0	0	2001–2005
Slaughter geese	172	14	8,1	2001
	168	3	1,8	2002
	180	9	5,0	2003
	134	6	4,5	2004
	140	3	2,1	2005
Total	794	36	4,5	2001–2005

Table 1. Continuation

Poultry	Flocks studied	Number of infected flocks	% infected flocks	Year
Ducks	64	7	10,9	2001
	42	0	0	2002
	46	3	6,5	2003
	35	1	2,85	2004
	31	0	0	2005
Total	218	11	6.8	2001–2005

Table 2. Salmonella serovars isolated from water fowl farms

Poultry	Year	Number of studied flocks	Flocks infected num./%	S. Typhi-murium num./ %	S. Enteri-tidis num. / %	S. Derby num. / %	S. Ana-tum num. / %	S. Dublin num. / %	S. Hadar num. / %	S. of group B num. / %	S. of group C num. / %	S. of group C_1 num. / %	S. of group C_2 num. / %	S. of group E num. / %
Geese	2001	211	15 (7,1)	2 (13,3)	10 (86,6)	1 (6,7)	0	1 (6,7)	0	1 (6,7)	0	0	0	0
	2002	203	3 (1,5)	2 (66,7)	0	0	0	0	0	1 (33,3)	0	0	0	0
	2003	223	9 (4,0)	5 (55,5)	2 (22,2)	0	0	2 (22,2)	0	0	0	0	0	0
	2004	165	6 (3,6)	1 (16,7)	3 (50,0)	1 (16,7)	0	0	0	0	0	1 (16,7)	0	0
	2005	169	3 (1,8)	2 (66,7)	1 (33,3)	0	0	0	0	0	0	0	0	0
Total	2001/ 05	971	36 (3,7)	12 (33,3)	16 (44,4)	2 (5,56)	0	3 (8,3)	0	2 (5,6)	0	1 (2,8)	0	0
Ducks	2001	64	7 (10,9)	0	2 (28,6)	0	0	0	0	2 (28,6)	1 (14,3)	0	1 (14,3)	1 (14,3)
	2002	42	0	0	0	0	0	0	0	0	0	0	0	0
	2003	46	3 (6,5)	0	1 (33,3)	0	0	0	1 (33,3)	0	0	1 (33,3)	0	0
	2004	35	1 (2,8)	0	0	0	0	0	1 (100,0)	0	0	0	0	0
	2005	31	0	0	0	0	0	0	0	0	0	0	0	0
Total	2001/ 05	218	11 (5,0)	0	3 (27,3)	0	0	0	2 (18,2)	2 (18,2)	1 (9,1)	1 (9,1)	1 (9,1)	1 (9,1)

E2 –
FEED HYGIENE – ANIMAL HEALTH – FOOD SAFETY

ORAL PRESENTATIONS

ANALYZING PRECONDITIONS FOR A TRANSPARENT MEAT PRODUCTION SYSTEM FROM FARM TO RETAIL FOCUSING ON ANIMAL HEALTH AND FOOD SAFETY

Breitenbuch, A.V., Meemken, D., Dickhaus, C.-P. and Blaha, T.

University of Veterinary Medicine Hannover, Field Station for Epidemiology, Bakum, Germany

SUMMARY

The paper describes a cooperative group of pig farmers, owning a slaughter plant operating an integrated system of pork production. In this system, a transparent production of pork leads to fulfilling the requirements of the new EU regulations for food safety (transparency, traceability, process optimization and risk-based decisions) and enables a feedback system from slaughterhouse to farm as a precondition for the continuous improvement of animal health and animal welfare in food producing animals and enhances the cooperation between all participants.

Keywords: transparent pork production, risk-based meat inspection, food chain information, improving animal health and animal welfare

INTRODUCTION

In a time of an increasingly rising number of “meat scandals”, many consumers ask themselves whether or not promises of meat producers regarding the quality and especially the safety of the meat can still be trusted. A cooperative group of pig farmers in Northern Germany uses means of communication, an integrated Veterinary Health Service and a vertically organized production system to enhance meat quality and safety by increasing animal health as well as animal welfare.

THE COOPERATIVE AND HOW IT WORKS

In the year 1974, a cooperative of around 250 sow herd owners with a production of about 420.000 piglets per year was founded. The target of this cooperation was the common use of strict quality and production guidelines as well as a shared marketing strategy for these piglets. Good genetic homogeneity and good animal health are achieved by the controlled acquisition of young sows and the controlled use of selected, stress-resistant boars.

As a logical consequence, the fattening pig herd owners using these piglets for producing slaughter pigs founded a cooperative including their own slaughterhouse as well with very strict rules in respect of feeding, housing conditions and a self established supervision system in the sense of self-control. This includes the supervision and consulting by agricultural advisers as well as by an integrated Veterinary Health Service.

Ways of communication between the slaughterhouse, the advisors and the veterinary practitioners are extremely short and efficient. This is due to the facts that the advisors are located in the slaughterhouse building and have the opportunity to see the results of the meat inspection directly at the slaughter line. The findings of the official meat inspection unit are registered online in a data processing system which is monitored by these advisors. The results of the meat inspection as well as the results of the carcass classification are submitted to the farms either by email or by mail, enabling the farmer to react very swiftly in accordance with the advisors and/or with the Veterinary Health Service. This service profits from the standardized conditions under which the pigs are fed and kept. It develops strategies for the prevention of diseases. Its veterinarians, who are highly specialized in pig herd management, try constantly to limit the use of drugs by optimising feed strategies and ventilation systems as well as other housing conditions. A close cooperation between agricultural advisors, farmers and the Veterinary Health Service is the basic requirement for the efficiency of this system.

The meat inspection unit uses the knowledge on the housing and feeding conditions of each individual herd, the use of drugs indicated by the "Animal Treatment Index" (Meemken and Blaha, 2007), the mortality rate, laboratory results like the Salmonella antibody herd-prevalence as food chain information. This information is the basic tool for the risk-based meat inspection. Food chain information, including the herd prevalence of organ findings of previously slaughtered pigs, enables the meat inspection unit to decide whether to increase or decrease the intensity of the inspection of the according batch of slaughter pigs of the same holding of provenance.

The Cooperative also cooperates very closely with the competent authorities, as shown in Figure 1.

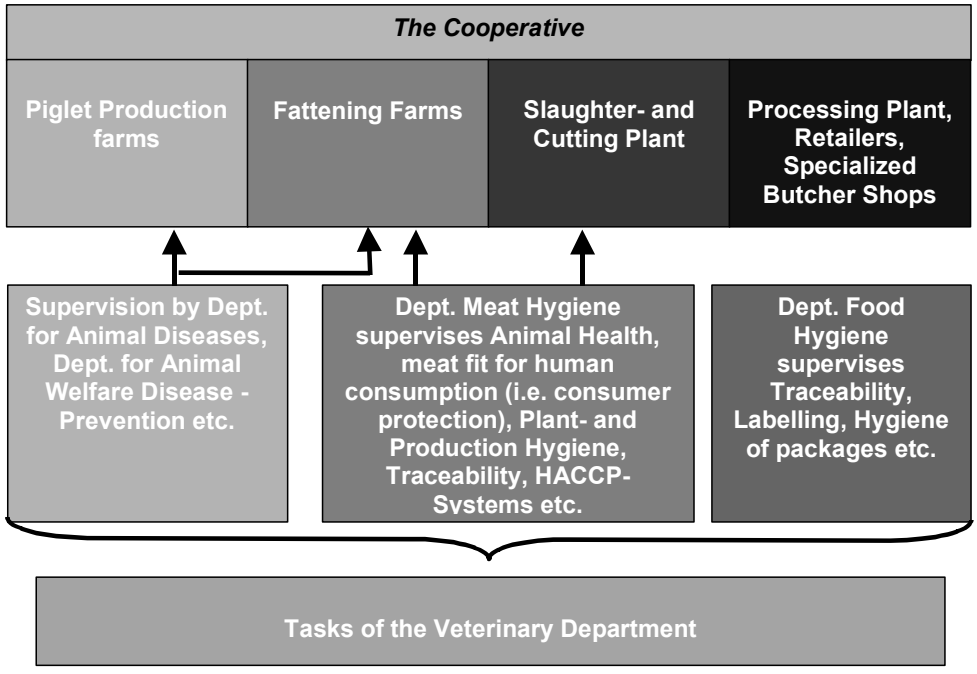

Figure 1. Cooperation between the State Veterinary Department and the Cooperative

ADVANTAGES OF THIS SYSTEM

The cooperation of a large number of farms enables the participants to acquire means of production (e.g. feed) collectively and thus gain profit. The feed comes from only three feed manufacturers and can therefore be influenced easily by the cooperative with respect to prescribing all components and ingredients. Constant sampling and control of the feed is easy and effective. Using only three suppliers makes also the required traceability easy. Attractive prices can be achieved on a basis of 50.000 tons of feed, negotiated biannually.

The work of the state meat inspection unit and, thus, the consumer protection becomes more efficient because all suppliers are known, and food chain information can be gained easily such as:

- Prevalence of organ findings in previous batches of the same supplier
- Herd health indicators such as mortality and drug use
- Salmonella monitoring results of previous batches of the same supplier
- Other laboratory results of previous batches of the same supplier
- The supplier's guarantee that the animals have not received drugs or medication with a withdrawal period greater than zero in a relevant period before slaughter

Besides that, these pieces of information are the basis of a proper risk-based meat inspection, enabling the meat inspection unit to decide about the intensity of the meat inspection for each batch of slaughter pigs.

CONCLUSIONS

Transparent meat production is possible. It is profitable for all participants of the described system. The system is not only enhancing food and consumer safety but also providing the means for a continuous improvement of the health and welfare in the animals that are kept and raised for food.

FOOD PROTEIN LEVEL DURING SOWS PREGNANCY

Kleisiari, M.

Agamemnonos 4, 40200 Elassona, Greece; E- mail: mdbouzoukis@yahoo.com

SUMMARY

The aim of this experiment is to see how many days before the end of pregnancy there is a need for more energy intake and for a higher content of protein in the sows diets.
Using two different diets in the two groups of pregnant sows the results show that we can feed the sows according to a corrected diet of 30 days before parturition and during the first 3 months of pregnancy an 11% level of protein in the diet is high enough to sustain a normal growth of the fetuses.

Keywords: gilt, corrected diet, deficient diet

INTRODUCTION

In pregnant sows the protein of the diet must cover sows needs for maintenance, for placenta formation and for the fetuses' growth. Protein is essential for life. Animal body gain is done of proteins, sows milk contents proteins. Surplus of protein in the diet is used as an energy source, in which case the cost of feeding becomes very high. At the same time protein surplus can cause kidney disease. Deficit of protein in the diet determines low performances and when it's under the maintenance needs it becomes dangerous for the health and the life of the animals.

From a practical point of view the most important hypothesis is that a special diet for pregnant sows in the second part of gestation can solve both requirements: supplementation of protein and more metabolic energy, as well. This way of acting is convenient especially for gilts.

Based on data concerning development and growth of fetuses it is possible to think that at the end of pregnancy there is need for more energy intake and for a higher content of protein in the sows' diets. That is the hypothesis of the present research.

MATERIALS AND METHODS

In order to test the former hypothesis I proposed to make an experiment with pregnant sows using two diets differing in their protein content. The first diet, the control one, called corrected (C), should have a 13% level of protein, as much as it is recommended by the literature. For the second diet, called deficient, (D), I decided to keep the 11% protein level as I met in a commercial pig reproduction farm. The reason of using in experiment a lesser percent of protein than recommended at the experimental diet is justified by the action of Mitsherliech's low of growth. According to this low the growing process follows a logistic curve and has to be expressed up to specific limits. Using a lower than recommended percent of protein in the experimental diet there is more chance to provoke a difference of the weight of piglets between the corrected and the uncorrected diets of the experiment.

So I have used for the two experimental lots the following receipts of powder combined feed:

Table 1. Structures of the corrected (C) diet and deficient (D) diet

	Corrected diet (C)				Deficient, uncorrected diet (D)			
Feed stuffs	%	TDN	M cal.	Protein	%	TDN	M cal.	Protein
Corn meal	55	44.0	189.4	440	55	44.0	189.4	440
Barley meal	30	22.8	89.8	300	30	22.8	89.8	300
Soya bean cake	10	7.8	31.5	440	5	3.9	15.8	220
Wheat bran	–	–	–	–	5	3.3	11.6	70
Meat meal	3	2.7	8.8	135	2	1.8	5.9	90
Minerals	2	–	–	–	3	–	–	–
TOTAL	100	77.3	319.5	1315	100	75.8	312.5	1120

In fact in this experiment I corrected feeding of sows by increasing the protein level of the diet from 11% to 13%. Food was given twice a day weighing 2kg of combined food per sow each time which means 4 kg of food per day. Straws were renewed twice a week in the sow's pen.

The experiment was located in a pig reproduction farm of 200 heads. Sows having the last mating in less than 21 days when the different feeding was implemented were not included in the experiment. The rest of the sows were grouped for 10 days interval from the last mating dividing each group in to parts, half of them received a correct 13% protein diet, the other half, i called the deficient lot, continued to receive the 11% protein diet. Altogether there were 10 groups of sows for each diet. Thus the time each group was fed in the experiment was different. The longest term was, as a mean, 100 days and the shortest one was of 10 days. The sows with less than 5 days of experimental feeding before farrowing were excluded from result evaluation. In order to have for each tens the same number of sows in the deficient and in the corrected feed lot one or two sows were excluded, just in case of data treatment, from one lot of the same ten. Finally, it has resulted the experimental design presented in Figure 1.

Days from mating

0–10	10–20	20–30	30–40	40–50	50–60	60–70	70–80	80–90	90–100	100–110	110–120	>120
		C	***D***	***C***	***D***	***C***	***D***	***C***	***D***	***C***	***D***	***C***
x	***x***	***5***	***4***	***8***	***8***	***8***	***3***	***3***	***4***	***6***	***8***	***x***
		D	***C***	***D***	***C***	***D***	***C***	***D***	***C***	***D***	***C***	
x	***x***	***5***	***4***	***8***	***8***	***8***	***3***	***3***	***4***	***6***	***8***	
		100	***90***	***80***	***70***	***60***	***50***	***40***	***30***	***20***	***10***	***<10***

Mean number of controlled feeding days

Figure 1. Experimental design concerning effect of two different levels of protein during pregnancy in sow

This experimental design gave me the possibility to appreciate: the effect of the two different levels of the protein in the diet and the term this effect takes place after. So the model must be treated as polyfactorial experimental design. It permitted to have equal number of cases in the both experimental groups of sows. That helps to apply statistical interpretation of data using analysis of variance.

RESULTS AND DISCUSSION

According to the primarily results, I have appreciated the body weight of piglets at birth and the suckling capacity of sows by the weight of the piglet lot at 21st day since birth. In this respect I have to say that after the farrowing all of the sows received the same diet containing 16% of protein and 77 TDN. Piglet weights at birth are shown in table 2.

Table 2. Body weight of piglets at birth

Time under exp.	**Lot**	**Statistics**				
		n	$\bar{x}$	s^2	s	V%
10	**C**	77	1.092	0.0202	0.142	13.0
	D	75	1.080	0.0232	0.152	14.0
20	**C**	63	1.358	0.0276	0.166	12.0
	D	58	1.174	0.0352	0.188	16.0
30	**C**	34	1.785	0.0837	0.289	16.0
	D	41	1.176	0.0359	0.189	16.0
40	**C**	23	1.587	0.0293	0.171	10.7
	D	20	1.425	0.0409	0.202	14.1
50	**C**	26	1.688	0.0522	0.228	13.5
	D	28	1.317	0.0593	0.243	18.5
60	**C**	103	1,502	0.1156	0.340	22.6
	E	97	1.205	0.0553	0.235	19.5
70	**C**	82	1.580	0.1040	0.323	20.4
	E	80	1.217	0.0609	0.246	20.2
80	**C**	81	1.508	0.0418	0.204	13.5
	E	74	1.266	0.0420	0.205	16.2
90	**C**	38	1.582	0.1026	0.320	20.2
	D	40	1.390	0.0578	0.240	17.2
100	**C**	39	1.682	0.1125	0.335	19.9
	D	39	1.230	0.1269	0.356	28.9

Sows fed on 11% protein level diet have born 552 piglets and the sows fed on a 13% protein level have born 566 piglets

Statistical analysis of data looks like that: Corrected feeding lot (C):

$n_C = 566$; $=1.505$; $s_C^2 = 0.07096$; $s_C = 0.266$; v%=17.7 Deficient, uncorrected feeding lot (D):

$n_D = 552$; $=1.266$; $s_D^2 = 0.05134$; $s_D = 0.227$; v%=17.9

The difference of means measures 0.239 kg, about 16% from the weight of piglets obtained from corrected feeding. In order to establish if such a difference is significant or not, I must apply Student's "*t"* test. In this case:

$$\sigma_t = \frac{552x0.05134 + 566x0.07096}{552 + 566 - 2} = \frac{68.5026}{1116} = 0.0613 \qquad \hat{\sigma} = \sqrt{0.0613} = 0.248$$

$$\hat{\sigma}_T = 0.248\sqrt{\frac{1}{552} + \frac{1}{566}} = 0.248x0.06 = 0.0185 \qquad t = \frac{0.239}{0.0185} = 12.92$$

Looking for this value of "*t*" on the Graphic of Student's at the site of over 100 degrees of freedom (in our case there are 1116 degrees of freedom) I noticed it is placed much higher the line of highly significant level. I can conclude for sure that an 11% protein level will determine that the body weight piglets to be born will be under the normal one.

Now, if I am looking at the differences in weight of piglets whose mothers were fed differently for the same length of time before parturition, (see table 2.), I notice the values of differences differ. But I have to find which of these differences are significant and which ones are not. In order to answer this question I formed a table of variance, presented as Table 3.

In this way I had the opportunity to judge the rate of contribution of the experimental factor to the total variance of the items and also to evaluate the significance of differences between different groups of piglets. It is possible to compare every group to any other group created by the experimental design (Table 3.).

The difference of means of body weight between the two groups of piglets whose mothers were fed differently for 10 days has no significance because 10 days is a too short term to show its influence on the corrected feeding.

Table 3. Analysis of variance table

Differences between groups of piglets within the same length of different feeding of their mothers

Days to part.	Number of piglets		s^2		Difference of $\bar{x}$	Counted *t*	Degrees of freedom	Tabulated *t* value			Significance
	C	D	C	D				.05	.01	.001	
10	77	75	.0202	.0232	0.012	0.50	150	1,96	2.576	3.291	–
20	63	58	.0276	.0352	0.184	5.73	119	1.98	2.617	3.373	+++
30	34	41	.0837	.0359	0.609	10.95	73	2.00	2.660	3.460	+++
40	23	20	.0293	.0409	0.162	2.85	41	2.02	2.704	3.551	++
50	26	28	.0522	.0593	0.371	5.76	52	2.00	2.617	3.375	+++
60	103	97	.1156	.0553	0.297	7.14	198	1.96	2.576	3.291	+++
70	82	80	.1040	.0609	0.363	8.01	160	1.96	2.576	3.291	+++
80	81	74	.0418	.0420	0.242	7.33	153	1.96	2.576	3.291	+++
90	38	40	.1026	.0578	0.192	3.00	76	2.00	2.660	3.460	+++
100	39	39	.1125	.1269	0.452	5.79	76	2.00	2.660	3.460	+++

For all other length of action the difference of means of similar groups in the two lots are highly significant, excepting the pair of groups with a 40 days action of the corrected feed where the difference of means was only significant.

In order to illustrate better the probable effect of the corrected feeding I applied the Analysis of Variance test.

Total variance = Σs^2 (all groups of both lots) = 0.7+0.5374=1.2374

Variance between groups = $\frac{o.7x566 + 0.5374}{566 + 552 - 2} = \frac{692.8448}{1116} = 0.62$ Variance inside groups = 1.2374–0.62=0.6174

It resulted that 50.1% of the total variance of the piglets' weight at birth was due to the corrected diet.

The same test applied to the last pair of groups' shows: **Total variance = 0.1125+0.1269=0.2394**

Between groups variance $= \frac{39x0.1125 + 39x0.1269}{39 + 39 - 2} = \frac{9.3366}{76} = 0.12$ Within groups variance = 0.2394–0.12=0.1194

The same result was obtained: 50.1% of the variance of the pair of groups receiving different feeding along all the gestation period pertains to these groups of variance.

There is no doubt than 11% of protein in the diet of the pregnant sows determines a significantly lower piglet weight at birth.

However how long the corrected diet must act before parturition in order to produce a significant difference between the mean body weight of piglets born after 100 days of corrected feeding and shorter terms.

Applying the Student's *t* test to appreciate the difference between the mean body weight of piglets born after a corrected feeding of pregnant sows during al pregnancy and the mean body weight of piglets born after 10 days corrected feeding before parturition I found:

$$\sigma^2 = \frac{39x0.1125 + 77x0.0202}{39 + 77 - 2} = \frac{4.3875 + 16.5627}{114} = 0.1838 \qquad \sigma = \sqrt{0.1838} = 0.4287$$

$$\hat{\sigma}_T = 0.4287\sqrt{\frac{1}{39} + \frac{1}{77}} = 0.4287\sqrt{0.0376} = 0.4287x0.1965 = 0.0842 \quad \bar{x}_{100} - \bar{x}_{10} = 1.682 - 1.092 = 0.59$$

$$t = \frac{0.59}{0.0842} = 7.00$$ **Degrees of freedom=39+77–2= 114**

The value of "*t*" for 114 degrees of freedom indicates a highly significant difference between the two means explained by the short term of action of the corrected diet.

Comparing in the same way the effect of a 100 days action of corrected feeding with the effect of 20 days corrected feeding I found:

$$\hat{\sigma}^2 = \frac{39x0.1125 + 63x0.0276}{39 + 63 - 2} = \frac{4.3875 + 1.7388}{100} = 0.0613 \quad \hat{\sigma} = \sqrt{0.0613} = 0.2475$$

$$\hat{\sigma}_T = 0.2475\sqrt{\frac{1}{39} + \frac{1}{63}} = 0.2475x\sqrt{0.0415} = 0.2475x0.2037 = 0.0504$$

$$\bar{x}_{100} - \bar{x}_{20} = 1.682 - 1.358 = 0.324 \quad t = \frac{0.324}{0.0504} = 6.429$$

The value of "*t*" for 100 degrees of freedom shows a highly significant difference between the mean body weights of these two groups of piglets. It means this term is also too short for corrected diet to show its effect.

When I compared the same group of 100 days corrected feeding piglets with the group of piglets whose mothers received corrected food for 30 days I found:

$$\hat{\sigma}^2 = \frac{39x0.1125 + 34x0.0837}{39 + 34 - 2} = \frac{4.3857 + 2.8458}{71} = \frac{7.233}{71} = 0.1019 \quad \hat{\sigma} = \sqrt{0.1019} = 0.3192$$

$$\hat{\sigma}_T = 0.3192\sqrt{\frac{1}{39} + \frac{1}{34}} = 0.3192x\sqrt{0.0534} = 0.3192x0.2311 = 0.074$$

$$\bar{x}_{30} - \bar{x}_{100} = 1.785 - 1.682 = 0.103 \quad t = \frac{0.103}{0.074} = 1.39 \qquad \text{Degrees of freedom} = 71$$

This time the value of *t* indicates, for 71 degrees of freedom, no significance of the difference between the mean body weights of the two groups of piglets.

It means that feeding sows on a corrected diet for 30 days before parturition I can have the same effect as feeding sows on corrected feeding along all the pregnancy. During the first 3 months of pregnancy 11% level of protein in the diet is high enough to sustain a normal growth of the fetuses. The same diet can be used both for adult sows and for gilts. Gilt growth doesn't claim higher level of protein.

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ANTIMICROBIAL RESISTANCE IN *CAMPYLOBACTER JEJNI* ISOLATED FROM BROILER CHICKENS IN ESTONIA DURING PERIODS 2002–2003 AND 2005–2006

Roasto, M.[1], Juhkam, K.[1], Tamme, T.[1], Praakle, K.[2], Hörman, A.[1] and Hänninen, M.-L.[2]

[1] Department of Food Science and Hygiene, Institute of Veterinary Medicine and Animal Sciences, Estonian University of Life Sciences, Estonia; [2] Department of Food and Environmental Hygiene, Faculty of Veterinary Medicine, University of Helsinki, Finland

SUMMARY

Our study was conducted in 2002–2003 and 2005–2006 to isolate campylobacters from a poultry production chain and determine the prevalence of antimicrobial resistance. All together we studied 167 broiler chicken *Campylobacter jejuni* isolates of Estonian origin. In 2002 and 2003 using a disc diffusion method and by E-test the resistance to ciprofloxacin, nalidixic acid, tetracycline, ampicillin, and erythromycin occurred in 44.4%, 44.4%, 22.2%, 19.4%, and 16.6% among the 36 *Campylobacter jejuni* isolates. We found no simultaneous resistance, of isolated strains, to three or more unrelated antimicrobial agents. Resistance to one or more antimicrobials was detected in 24 isolates (66.7%). None of the chicken isolates were resistant to gentamicin. In 2005 and 2006 a total of 131 *C. jejuni* isolates were collected over a 13-month period and the MICs were determined. Resistance to one or more antimicrobials was detected in 104 isolates (79.4%). A high proportion of the isolates were resistant to enrofloxacin (73.3%) and nalidixic acid (75.6%). Multidrug resistance (to three or more unrelated antimicrobials) was detected in 36 isolates (27.5%), all of which were resistant to enrofloxacin. Our results showed that multidrug resistance was significantly associated with enrofloxacin resistance ($p<0.01$) and the use of enrofloxacin may select multiresistant strains.

Keywords: broiler chicken, *Campylobacter jejuni,* susceptibility, multiresistance

MATERIALS AND METHODS

Isolates

We studied 36 broiler chicken *Campylobacter* isolates from 396 raw broiler chicken meat samples obtained from retail stores in Estonia between January 2002 and December 2003. In 2005 and 2006 our study included 105 *Campylobacter* strains analysed from a total of 1254 fresh faecal samples at an Estonian chicken farm and from 264 chicken caecal contents at slaughterhouse level. Furthermore, 26 isolates from 340 randomly purchased fresh chicken meat samples at the retail level in Estonia were analysed. All 167 isolates were identified as *C. jejuni*.

One loopful (10 µl) of faecal material or intestinal contents from the caecum was taken, and the material was transferred into tubes containing 10 ml of Preston enrichment broth (Oxoid; Basingstoke, Hampshire, England). The tubes with enrichment broths were stored at 4ºC and transported immediately to the laboratory. Enrichment broths were incubated at 42 ± 0.5ºC for

24 h in microaerobic conditions. Analyses for campylobacters were carried out at the State Veterinary and Food Laboratory, Tartu, Estonia.

Fresh poultry meat samples were analysed for campylobacters using the method of Nordic Committee on Food Analysis (Anonymous, 1990), which includes an enrichment phase in Preston broth. Briefly, 250 ml of Preston enrichment broth (Oxoid; Basingstoke, Hampshire, England) was added to a 25-g of meat sample, and the sample was stomached for 60 s. The enrichment broth was incubated at 42 ± 0.5 ºC for 24 h in microaerobic conditions.

After 24 h incubation a loopful of the enrichment broth both from faecal and meat samples was plated on modified charcoal cefoperazone deoxycholate agar (mCCDA) (Oxoid; Basingstoke, Hampshire, England) and examined for typical growth after 48 h microaerobic incubation. Organisms growing on mCCDA plates were streaked on Brucella blood agar (Oxoid) and confirmed by Gram staining, motility analysis and oxidase and catalase tests as campylobacters. One randomly chosen colony from each positivesample was analysed for hippurate hydrolysis, and hippurate-positive isolates were regarded as *C. jejuni*. After the original isolation, the strains were stored at –70 °C in glycerol broth (15% [vol/vol] glycerol in 1% [wt/vol] proteose peptone).

Antimicrobial susceptibility testing

In 2002 and 2003 all *Campylobacter* isolates were tested by disc diffusion method against ampicillin (25 µg), ciprofloxacin (5 µg), erythromycin (15 µg), gentamicin (10 µg), nalidixic acid (30 µg), and tetracycline (10 µg) (Oxoid), and by the E-test (AB Biodisk, Solna, Sweden) against ampicillin, ciprofloxacin, erythromycin, and tetracycline.

Campylobacter isolates were first grown on blood agar plates and were transferred in 5 ml of Mueller-Hinton (MH) broth (Oxoid) and incubated at 37°C for 24 h in microaerobic conditions. Inoculum from the MH broth was diluted and a turbidity equivalent of a 0.5 McFarland standard was adjusted in physiological peptone-saline water and the growth suspension was spread on the MH blood agar plates (Oxoid, supplemented with 7% horse blood), the disks or E-test strips containing antimicrobial compounds were laid on the plates. The plates were incubated at 37 °C for 24 h in microaerobic conditions. The diameter of the growth inhibition zone was measured according to the CLSI (2004). MIC values were determined by E-test according to the instructions given by the manufacturer (AB Biodisk). *C. jejuni* 143483 was used as control strain in the antimicrobial susceptibility testing.

The following zone diameter (mm) and MIC breakpoints for resistance were applied: ampicillin ≤ 13 mm and MIC $\geq$ 32 µg/ml, ciprofloxacin ≤ 26 mm and MIC $\geq$ 4 µg/ml, erythromycin ≤ 26 mm and MIC ≥ 32 µg/ml, gentamicin ≤ 12 mm, nalidixic acid ≤ 26 mm, and tetracycline ≤ 31 mm and MIC ≥ 16 µg/ml (Anonymous, 2004; CLSI, 2004).

In 2005 and 2006 all 131 *C. jejuni* isolates were tested for minimal inhibitory concentration (MIC) by a broth microdilution method (National Veterinary Institute, Uppsala, Sweden) against ampicillin, enrofloxacin, erythromycin, gentamicin, nalidixic acid and oxytetracycline. The MIC-based microdilution was carried out at the laboratory of the Department of Food Science and Hygiene of the Estonian University of Life Sciences, Tartu, Estonia. *Campylobacter* isolates were first cultured on Brucella blood agar (Oxoid, Basingstoke, Hampshire, England) and incubated at 37 °C for 48 h. A loopful (1µl) of bacterial growth was transferred to 10 ml of cation-adjusted Mueller-Hinton (CAMHB) broth (Oxoid, Basingstoke, Hampshire, England) and then incubated at 37 °C for 24 hours to achieve a level of around 10^8 CFU/ml. The bacterial suspension was diluted to 10^6 CFU/ml. One hundred microliters (µl) of bacterial suspension was inoculated into each well of microtitre plates. The plates were incubated at 37 °C for 40 h in microaerobic

conditions. The MIC was read as the lowest concentration completely inhibiting visible growth of campylobacters in accordance with the instructions given by the test manufacturer (National Veterinary Institute, Uppsala, Sweden). Control of the purity of the bacterial suspension was carried out by plating 10 µl of bacterial suspension on Brucella agar. The density of the bacterial suspension was controlled according to the guidelines of the Estonian Veterinary and Food Laboratory, and colony counts from 50 to 250 per plate were accepted (Anonymous, 2004b and Anonymous 2005b). *C. jejuni* ATCC 33560 was used as a control strain in the antimicrobial susceptibility testing. The following MIC breakpoints for resistance were applied: ampicillin 32 µg/ml, enrofloxacin 1 µg/ml, erythromycin 16 µg/ml, gentamicin 8 µg/ml, nalidixic acid 32 µg/ml and oxytetracycline 4 µg/ml (Anonymous, 2004b and Anonymous 2005b).

Statistical analysis

All individual results were recorded using MS Excel 2003 software (Microsoft Corporation; Redmond, WA, USA), and the statistical analysis was performed with the Statistical Package for Social Sciences 13.0 for Windows (SPSS Inc.; Chicago, IL, USA). Non-parametric Spearman's rank order correlation coefficients with two-tailed p-values and odds ratios (ORs) were calculated for bivariate cross-correlations between resistances to the six antimicrobials analysed as well as between antimicrobials and multiresistance, which was defined as resistance to three or more unrelated antimicrobials simultaneously. Furthermore, a non-parametric Mann-Whitney independent samples test was conducted to compare the level of antimicrobial resistance between multiresistant and non-multiresistant strains.

RESULTS

In 2002 and 2003 by disc diffusion method the resistance to ciprofloxacin, nalidixic acid, tetracycline, ampicillin, and erythromycin occurred in 44.4%, 44.4%, 22.2%, 19.4%, and 16.6% of the isolates (n = 36). All isolates were susceptible to gentamicin. Isolates with resistance to ciprofloxacin were also resistant to nalidixic acid. Resistance of isolates to two unrelated antimicrobials was mainly to a combination of ciprofloxacin/nalidixic acid and tetracycline (8/36, 22.2%). Three isolates showed a resistance combination of ampicillin and erythromycin, and two isolates of ampicillin and ciprofloxacin/nalidixic acid. We found no simultaneous resistance, of isolated *Campylobacter jejuni* strains, to three or more unrelated antimicrobial agents in 2002 and 2003. Results of disk diffusion method and the E-test were similar and all isolates resistant or susceptible by the disk diffusion method showed the same results by E-test.

In 2005 and 2006 resistance to one or more antibiotics was detected in 104 isolates (79.4%). Twenty isolates (15.3%) were resistant to three unrelated antimicrobials, thirteen isolates (10%) to four unrelated antimicrobials and three isolates (2.3%) to all tested antimicrobials. Enrofloxacin and nalidixic acid were regarded as one group of antimicrobials. Resistance of isolates to three unrelated antimicrobials was mainly to a combination of enrofloxacin/nalidixic acid, erythromycin and oxytetracycline (4.6%). Resistance of isolates to four unrelated antimicrobials was mainly to a combination of enrofloxacin/nalidixic acid, erythromycin, gentamicin and oxytetracycline (8.4%). Three isolates were resistant to five unrelated antimicrobials, comprising a combination of ampicillin, enrofloxacin/nalidixic acid, erythromycin, gentamicin, and oxytetracycline (2.3%). The highest frequency of resistance was to nalidixic acid and enrofloxacin (75.6% and 73.3%, respectively), followed by oxytetracycline (32.1%), erythromycin (19.8%),

gentamicin (19.1%) and ampicillin (7.6%). Multidrug resistance (to three or more unrelated antimicrobials) was significantly ($p<0.01$) associated with enrofloxacin and nalidixic acid resistance. The level of antimicrobial resistance was higher for nalidixic acid in multiresistant *C. jejuni* strains than in non-multiresistant strains (Mann-Whitney test, $p=0.026$), while resistance for other antimicrobials was not statistically different ($p>0.05$) between multi- and non-multiresistant strains.

DISCUSSION

An important finding of our study in 2002 and 2003 was the recognition of a high number of *Campylobacter* isolates with increased antimicrobial resistance to ciprofloxacin, 44.4% (16 isolates MIC $\geq$ 32 μg/ml). Enrofloxacin and flumequine, both fluoroquinolone group antimicrobials, are accepted for poultry treatment in Estonia (Anonymous, 2005), possibly explaining the high level of resistance detected among Estonian isolates.

Ampicillin is a widely used antimicrobial in veterinary medicine and from this group the amoxicillin is accepted for use in veterinary medicine in Estonia (Anonymous, 2005). Resistance to ampicillin in Estonian broiler isolates was 19.4%.

The resistance of Estonian *Campylobacter* isolates to erythromycin was 16.6%. None of the chicken isolates showed resistance to gentamycin in our study performed in 2002 and 2003.

In the period of 2005 and 2006, an important finding was the high number (79.4%) of antimicrobial-resistant *Campylobacter jejuni* isolates, 36 (27.5%) of which exhibited multiresistance (resistance to three or more unrelated antimicrobials). Resistance was especially high to enrofloxacin (80 isolates MIC $\geq$ 4 μg/ml). In the present study, two different fluoroquinolones were studied. Cross-resistance between the different fluoroquinolones has been previously documented (Rautelin et al., 2003; Griggs et al., 2005), as their modes of action are similar (inhibition of DNA gyrase). Most of the *Campylobacter* strains for which enrofloxacin MICs were high were also not inhibited by low concentrations of nalidixic acid. Gentamicin and erythromycin resistance was rather high among our *C. jejuni* strains 19% and 19.8%, respectively. The reason for this is unknown but could be associated with the veterinary use of latter antimicrobial agents in broiler chicken production. High MICs of both macrolides and fluoroquinolones for isolates pose a problem and because erythromycin is considered as a first-line choice of treatment for human *C. jejuni* infections, this resistance has an important public health impact. We found a high proportion of multidrug-resistant isolates (27.5%); all of these were resistant to enrofloxacin and all except one resistant to nalidixic acid. Our results showed that multidrug resistance was significantly associated with enrofloxacin and nalidixic acid resistance (correlation coefficient 0.372 and 0.310, $p<0.01$). These findings suggest that the use of fluoroquinolones may select multiresistant strains since resistance to erythromycin, gentamicin or oxytetracycline was exceptional without simultaneous resistance to fluoroquinolones. In conclusion, multidrug resistance in Estonian broiler chicken isolates was one of the highest reported in studies of broiler chicken *Campylobacter* isolates. The widespread emergence of multiresistant isolates poses a threat to humans and limits therapeutic medication. The European ban of the antimicrobial growth promoters (came into force since 01.01.2006 in Estonia) should be strictly followed. It will be important to monitor the trends in resistance after the withdrawal and over time. In Estonia, more restricted use of antimicrobial agents, especially fluoroquinolones, in food animal production should be implemented.

ACKNOWLEDGEMENTS

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COMPARATIVE EVALUATION OF DIFFERENT ELISA SYSTEMS FOR *INTRA VITAM* DIAGNOSIS OF PORCINE *SALMONELLA* INFECTION CAUSED BY *S.* TYPHIMURIUM AND *S.* INFANTIS

Roesler, U.*, Matthies, C., Szabo, I., Schürer, K., Nöckler, K., Hensel, A. and Truyen, U.

Institute of Animal Hygiene and Veterinary Public Health, An den Tierkliniken 1, 04103 Leipzig, Germany; E-mail: roesler@vetmed.uni-leipzig.de

SUMMARY

Salmonellosis is one of the most important enteric infections in man and in livestock. Various serotypes of *Salmonella enterica* can cause a variety of clinical and subclinical infections, which are mainly self-limiting gastroenteritis or systemic diseases. Beside *Salmonella* (*S.*) Typhimurium, *S. Derby* and *S. infantis* are the most important cause of porcine *Salmonella* infections. Although pigs usually do not develop clinical salmonellosis, they become carriers and shedders resulting in a substantial disease-causing potential for humans via meat and faeces.

Salmonella infections can be directly diagnosed in the piggery or at the slaughterhouse by isolating salmonellae with various established cultural methods or by serodiagnosis using lipopolysaccharide-based ELISA-systems or a whole-cell-lysate based standard ELISA test. These serological results are used to classify pig herds in one of three categories. Category 3 has the highest prevalence of *Salmonella* infection, defined as at least 40 percent of the pigs examined being seropositive. Category 2 herds have a moderate number of antibody-positive pigs, whereas, herds of category 1 have no or only a low prevalence of antibody-positive pigs.

The object of this study was the comparative evaluation of four indirect *Salmonella* ELISA tests approved in Germany to detect *Salmonella* infection of pig caused either by *S. typhimurium* or *S. infantis*. Three tests are based on a LPS-antigen and directed against specific IgG antibodies. The fourth test is based on a purified *S. typhimurium* whole-cell-lysate antigen and discriminates between *Salmonella* specific IgM-, IgA-, and IgG- antibodies. In a longitudinal study 6 weeks old hybrid piglets were orally infected with *S.* Typhimurium or *S. infantis*. During an observation period of 120d clinical and bacteriological parameters were weekly monitored and serum samples were in parallel investigated by the respective ELISA methods.

The results of the evaluation of the ELISA tests (sensitivities) are presented. It became obvious that the tested LPS-ELISA systems failed to detect *S. infantis* infected pigs (which shed the pathogen in high amounts throughout the study) until day 90 after infection, whereas, the whole-cell-lysate ELISA detected significant more *S. infantis* infected pigs in the early stage of infection. In contrast, all investigated ELISA-systems detected the majority of the *S. typhimurium* infected pigs beginning at day 24 post infection.

EFFICACY OF SEQUESTRANT/CHELATOR AMADÉITE, IN THE BINDING OF MYCOTOXINS DURING TRANSIT THROUGH A DYNAMIC GASTROINTESTINAL MODEL (TIM) SIMULATING THE GI CONDITIONS OF PIGS.

Demais, H.[1] and Havenaar, R.[2]

[1] OLMIX S.A, Z.A du Haut du Bois, 56580, BREHAN, France, hdemais@olmix.com;
[2] TNO. The Netherlands

ABSTRACT

Objective

The objective of this study was to investigate the efficacy of a pillared interlayer clay Amadéite on the binding of various mycotoxins and consequently to inhibit the availability for absorption of mycotoxins during gastro-intestinal transit of the TNO in vitro dynamic gastrointestinal model, the TIM-1 system.

MATERIEL AND METHODS

The experiments in TIM-1 were performed under the average physiological conditions of the gastrointestinal tract of young adult pigs after the intake of a solid pig meal artificially contaminated with the mycotoxins deoxynivalenol (approx. 1 ppm), and fumonisin (approx. 2 ppm).

The feed and pooled dialysate samples were analysed on the concentrations of the two mycotoxins (DON, Fumonisin). The difference in absorbed amounts between the control experiment (0% level of adsorbent) and the experiments with 0.01% and 0.1% adsorbent added to the pig feed, determines the efficacy of the mycotoxin-binding in inhibiting mycotoxin absorption.

The feed and dialysate samples of each TIM-run are analysed on some specific nutrients:

- Nitrogen Kjehldahl analysis (to determine the protein digestibility);
- Free glucose; (to determine the carbohydrate digestibility);
- Vitamin B1 (thiamine) and B2 (flavine dinucleotide) (as example-vitamins to determine the bioaccessibility of water soluble vitamins).

The difference in absorbed amounts of nutrients between the control experiment (without adsorbent) and the experiments with two levels of adsorbent/chelator, determines the potential binding effect of the sequestrant on the feed nutrients.

RESULTS/CONCLUSION

The results showed that Amadéite has a binding capacity for fumonisin, even at the low dose of 0.01% in pig feed, contaminated with two different mycotoxins at the levels of 0.8 to 2 ppm. It

inhibits the bioaccessibility of fumonisin with approximately 50% (0.01% level of Amadéite) to 60% (0.1% level).

Besides fumonisin, Amadéite also inhibits the bioaccessibility of deoxynivalenol (DON). Added to the contaminated pig feed at the level of 0.1%, the inhibition was approximately 40% in comparison to the control without Amadéite.

Previous studies in the TIM system with activated carbon demonstrated a reduction in the bioaccessibility of DON of 30–40% in comparison to the control experiment (Avantaggiato et al., 2004). However, the level of activated carbon in the feed ranged from 0.5% up to 2%.

The absorbance capacity of Amadéite did not inhibit the digestibility of proteins and carbohydrates as shown by an unchanged bioaccessibility of nitrogen and glucose, respectively.

The addition of Amadéite to the pig feed at the level of 0.1% showed a 30% increase in the bioaccessibility of vitamin B1 in comparison to the control, but did not change the bioaccessibility of vitamin B2.

ANTIBIOTIC RESISTANCE IN SWISS VEAL CALVES AT SLAUGHTER

Di Labio, E.[1], Regula, G.[1], Miserez, R.[2] and Ledergerber, U.[1]

[1] Federal Veterinary Office, Schwarzenburgstr. 155, CH-3003 Bern, Switzerland;
[2] National Centre for Zoonoses, Bacterial Animal Diseases and Antimicrobial Resistance, Institute of Veterinary Bacteriology, Vetsuisse Faculty, Laenggass str. 122, CH-3012 Bern, Switzerland.

SUMMARY

Resistant bacteria can be transferred from animals to humans and may compromise antimicrobial treatment in case of infection. Among bacteria isolated from veal calves resistance was mainly observed to antibiotics with high use in food animals. However, a rather high number of synercid-resistant *E. faecium* and ciprofloxacin-resistant *Campylobacter* were detected. Four *Campylobacter* strains exhibited resistance to ciprofloxacin, erythromycin and tetracycline. Calf purchase, large finishing groups, outdoor access, feeding of milk by-products and administration of antibiotics through feed upon arrival of the animals on the farm significantly increased the risk of antibiotic resistance at farm level.

Keywords: calves, *E. coli*, *Enterococcus* spp., *Campylobacter* spp., antibiotic resistance, risk factors

INTRODUCTION

The use of antimicrobial agents in food animals can select for resistant pathogenic and commensal bacteria in these animals. Through direct contact or the consumption of contaminated food resistant bacteria or resistance genes can be transmitted to humans and may hinder a successful treatment in case of infection. Due to the frequent use of antibiotics in the raising and finishing of calves and due to the possible feeding of milk which contains antimicrobial residues, veal calves have to be considered as a high-risk population for resistant bacteria. In order to ensure food safety, it is important to monitor the resistance situation in zoonotic agents and indicator bacteria in calves at slaughter. By identifying possible risk factors for antibiotic resistance at farm level specific measures can be taken to improve the management and reduce the usage of antibiotics and thereby the risk of resistance. The objectives of this project were i) to determine the prevalence of antibiotic resistance in *E. coli*, *Enterococcus* spp. and *Campylobacter* spp. in calves at slaughter, and ii) to identify possible risk factors at farm level associated with the occurrence of resistance.

MATERIAL AND METHODS

Faecal samples from 500 randomly selected calves originating from 129 farms were collected at slaughter. The samples were cultured for *E. coli*, *Enterococcus* spp., and *Campylobacter* spp. For antimicrobial susceptibility testing, the MIC (minimal inhibitory concentration) technique by the

broth microdilution method was performed as recommended by the Clinical and Laboratory Standards Institute (CLSI). Resistance was defined following the breakpoints published in approved literature (ARBAO-II 2005, CLSI M7-A6 and M100-S15, DANMAP 2004 and FDA 2002). From 100 farms, data on farm management, animal husbandry and antibiotic treatments of the calves were collected by questionnaire. Risk factors at farm level associated with resistance against selected antibiotics were identified by logistic regression.

RESULTS

E. coli were isolated from 467 out of the 500 faecal samples (93.4%). Of those, 321 strains (68.7%) were resistant to at least one of the tested antibiotics. Resistance was most frequently observed to sulfamethoxazole (64% of the isolates), tetracycline (56.5%), streptomycin (53.1%) and ampicillin (47.5%). All isolates were susceptible to ceftiofur and colistin. Prevalence of resistance in *E. coli* is shown in Figure 1. About 60% of the *E. coli* isolates were resistant to two or more antibiotics, most frequently to ampicillin/ neomycin/ streptomycin/ sulfamethoxazole and tetracycline.

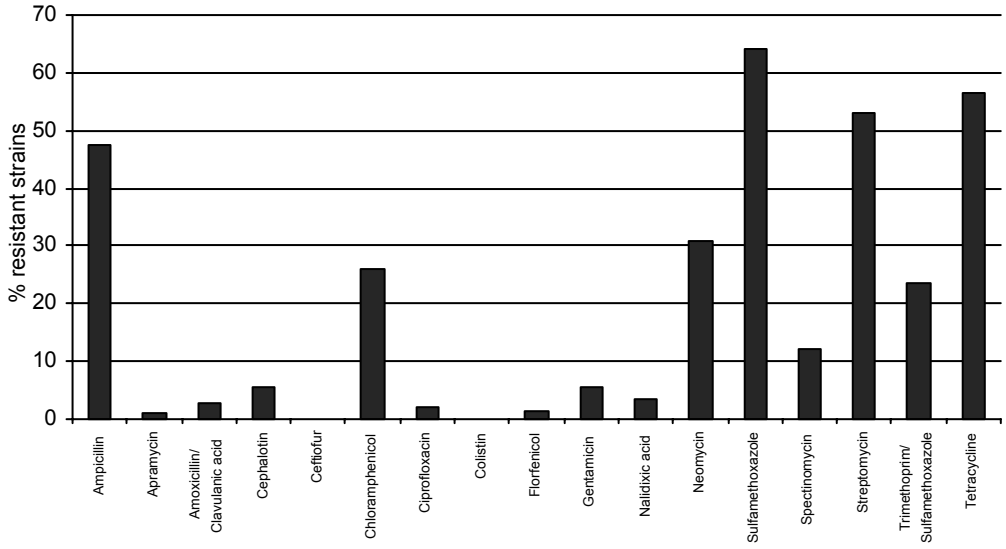

Figure 1. Prevalence of resistance in *E. coli* (n=467) from veal calves

In total, 413 *Enterococcus* strains were isolated from 359 (71.8%) out of the 500 faecal samples. Of those, 195 strains were typed as *E. faecalis* and 160 as *E. faecium*. All *E. faecium* and 99.5% of the *E. faecalis* isolates were resistant. The resistance pattern differed between the two species (Figure 2).

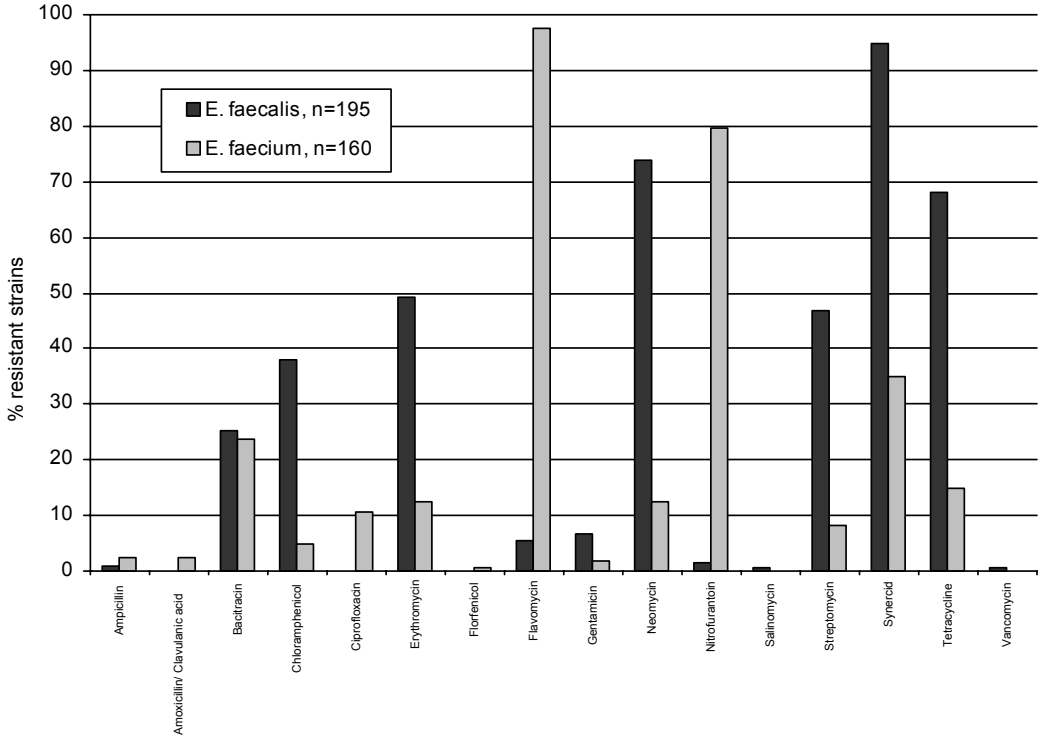

Figure 2. Prevalence of resistance in *E. faecalis* and *E. faecium* from veal calves

Campylobacter spp. were isolated from 202 (40.4%) out of the 500 faecal samples and 137 strains (67.8%) showed resistance to at least one of the tested antibiotics. Frequent resistance was observed to nalidixic acid (51.0% of all *Campylobacter* isolates), tetracycline (39.6%), ciprofloxacin (33.7%) and streptomycin (21.8%). No resistance to amoxicillin/ clavulanic acid, florfenicol and meropenem was detected. The 27 strains identified as *C. coli* showed resistance more often than the 129 *C. jejuni* isolates (88.8% and 54.3% of the isolates, respectively). Prevalence of resistance in *C. jejuni* and *C. coli* is shown in Figure 3. Multidrug resistance was more frequently observed in *C. coli* than in *C. jejuni* (63% and 38.8%, respectively). Two *C. coli* and two *C. hyointestinalis* with multiple resistance exhibited resistance to ciprofloxacin, erythromycin and tetracycline. No erythromycin-resistant *C. jejuni* was found.

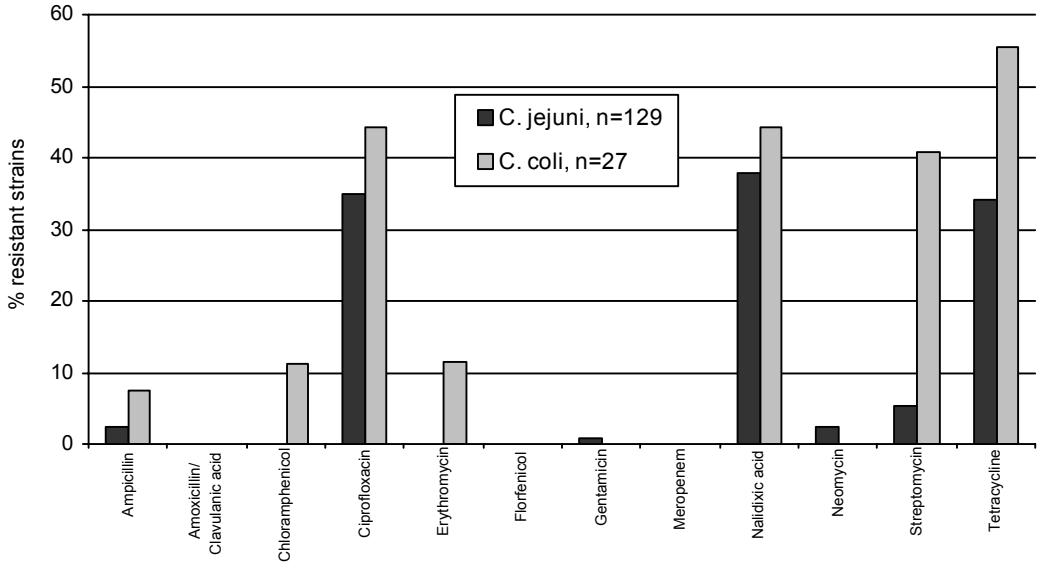

Figure 3. Prevalence of resistance in *C. jejuni* and *C. coli* from veal calves

The analysis of possible risk factors for increased antibiotic resistance at farm level revealed significant effects for calf purchase, large finishing groups, outdoor access, feeding of milk by-products and administration of antibiotics through feed upon arrival of the animals on the farm. Protective effects were obtained for production with specific regulations, i. a. restrictions in antimicrobial usage, and for administration of antibiotics by injection. The possible feeding of milk containing antimicrobial residuals showed no effect on the occurrence of resistance.

DISCUSSION

Resistance was frequently observed to antibiotics with high use in food animals such as tetracycline, streptomycin, penicillins and sulfonamides. Resistance to antibiotics used for human therapy was generally low. Only one vancomycin-resistant *Enterococcus* strain was found. However, a relatively high number of *E. faecium* isolates showed resistance to synercid, an antibiotic primarily used for the treatment of vancomycin-resistant *E. faecium*-infections in humans. About one-third of the *Campylobacter* isolates showed resistance to ciprofloxacin and four *Campylobacter* strains with multiple resistance exhibited resistance to ciprofloxacin, erythromycin and tetracycline, the antibiotics most frequently used for the treatment of human campylobacteriosis. However, *C. jejuni*, the *Campylobacter* species most frequently involved in human illness, was not among these multiresistant strains. Calf purchase, large finishing groups, outdoor access and feeding of milk by-products increased the risk for antibiotic resistance probably by having an influence on calf health and thereby an indirect impact on antibiotic use.

In conclusion, the study showed that veal calves may serve as a reservoir for resistant bacteria. However, most observed resistance prevalences were comparable to the prevalences found in bacteria from other Swiss food animals at slaughter (Anonymous, 2007). Nevertheless, calf

husbandry should be optimized not only with respect to animal welfare, but also to animal health and food safety. By improving farm management, the usage of antibiotics and thereby the risk of resistance can be reduced.

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THE INFLUENCE OF FISH OIL SUPPLEMENTATION TO THE FEED RATION OF COWS ON THE LEVEL OF CHOLESTEROL AND ITS FRACTION IN THEIR BLOOD SERUM[3]

Korczyński, M., Pogoda-Sewerniak, K., Janeczek, W. and Kupczyński, R.

Wroclaw University of Environmental and Life Sciences, Institute of Animal Hygiene and Ichthyology, ul. Chełmonskiego 38c, 51–630 Wroclaw, Poland

SUMMARY

The aim of the searching was to define the influence of fish oil supplement to the feed ration of high producing cows on the level of cholesterol and its fraction in their blood serum. The research was carried out on primiparous cows (1 – without supplementation, 2 – fish oil supplementation) as well as on multiparous cows (3 – without supplementation, 4 – fish oil supplementation) in the period from the 10th day till the 8th week after calving. Cows were given, together with the feed ration (TMR system), fish oil in amount of 1% of daily ration of dry matter, during all the experiment period. At the first day of experiment and after 4, 6 and 8 weeks, level of cholesterol and its fraction in the blood serum of examined cows has been signified.

On the basis of received results an increase of total cholesterol by all examined individuals and what follows, increase of its fraction, has been stated. By the primiparous cows of control group the increase was highest at the end of the experiment – 130% in relation to the initial value and by the individuals receiving 1% of fish oil in the feed ration, only 18%. By multiparous cows, receiving the supplementation, twice as small increase of LDL fraction concentration in relation to the control individuals has been observed.

Keywords: cows, fish oil, cholesterol, HDL, LDL, blood serum

INTRODUCTION

Modification of fatty acids composition in the cow diet is one of few methods that enable gaining of milk with higher content of multiunsaturated fatty acids, important from dietary point of view [3, 10]. The content of individual fatty fractions in cow milk depends on feeding and ipso facto from fodders applied in food ration. Considering the fact, that in the case of high productive cows, TMR system is recommended, advisable would be modification of milk composition, which can be obtained applying fish oil, receiving from liquid productive raw materials form sea fish processing.

Separate problem are metabolic transformations, therein energetic, by milk cows. In the perinatal period and at the beginning of lactation it comes to violent increase of energetic demand and nutritional components requirements, which are necessary for milk production, with

[3] The research was carried out within the framework of the searching project, financed by the found of MNiSW, project number: 2P06Z 060 027

simultaneously hormonal changes assuring homeostatic subordinating of the organism to the lactation process [15]. The energetic demand, especially by high productive cows, exceeds usually in the beginning of lactation energy volume consumed from the feed. Then negative energy balance and over-lipolysis of spare fat occurs [2, 7]. In consequence it can come to expansion of sub clinical or clinical ketosis and liver lumbering [7, 15]. The energy volume in the dose, without proportions change of nutritive fodder to bulky feed, can be increased among other things through addition of fats. Great interest for this nutrient in the last year's results from difficulties in meeting energetic demands of high productive cows in the first lactation phase. Moreover, using of fodder fat in cow feeding causes improvement of ovaries operation, lesser mortality of embryos, influencing profitably on the reproduction rate [12]. According to Hagemeister and Voigt [8] the fodder fat is more effectively used to meet energetic demands, connected with milk production, than fat of the organism. As Goff and Horst [6] show, high energy consume and its optimal transformations by cows in initial lactation have essential influence on the health and productivity of high productive milk cows.

The aim of searching was application of fish oil by feeding of high productive cows as well as its influence on carbohydrate- and lipid transformations – level of total cholesterol and its fractions HDL and LDL.

MATERIAL AND METHODS

The investigation was conducted on dairy cows farm numbering 250 animals, crossbreeds cb x hf of more than 75% of hf breed genes, featuring milk yield for previous lactation 8500 kg. The cows were kept in loose barn and fed according to TMR system (total mixed rations). The fodders used for cow feeding (TMR components) were subjected to chemical analysis in Blattin Laboratory in Langenfeld to determine the content of dry mass, total protein, raw ash, raw fat, raw fiber and its fractions (ADF and NDF), as well as mineral components: calcium, phosphorus, magnesium and sodium [2]. On the basis of the analysis done, there were worked out food rations for cows according to DLG norms [5]. Quantitative composition and food value of the doses were shown in Table 1.

Table 1. Content and nutritional composition of the cows' diets

Dose composition	Units		
Maize silage	kg	25,000	
Fresh pressed pulp		8,000	
Maize seed silage		5,500	
Barley		3,000	
Soybean		2,700	
Rapeseed		2,500	
Barley strain		2,000	
Sodium bicarbonate		0,200	
Premix		0,180	
Forage chalk		0,150	
Fat-mineral preparation*		1,126	
Nutritional composition		Control groups I and II – (primiparous and multiparous)	Experimental groups III i IV (primiparous and multiparous)
Dry mass	%	48,17	49,06
Raw fibre % dry mass	% s.m.	14,03	13,65
NEL	MJ/kg s.m.	6,87	8,24
Total protein	g/kg s.m.	3858,00	3864,59
Available total protein in small intensive	g/kg s.m.	3806,63	3813,19
Ca	g/kg s.m.	6,95	6,75
P	g/kg s.m.	4,36	4,35
Na	g/kg s.m.	1,40	1,35
Mg	g/kg s.m.	2,54	3,19

*preparation supplementation in experimental groups (III i IV)

40 clinically healthy cows were selected for strict examination and, following the analogy method, considering the sequence of lactation (primiparous and multiparous in 2 or 3 lactation, as well as milk yield for previous lactation); they were at random classified into 4 equipotent groups. The factor differentiating particular groups was fat-mineral preparation (F–M), additionally administered for 8 weeks twice a day in the dose of 563 g per head – fish oil amounted 1% of dry mass ration. The layout for this experiment was shown in Table 2.

Table 2. The scheme of the experiment

Cows	Feed groups			
	I – control	II control	III – experimental	IV – experimental
Primiparous	No supplement	–	supplement – fish oil (1% of dry DM)–	–
Multiparous	–	– No supplement	–	supplement – fish oil (1% of DM)

F-M preparation contained fish oil 22%, bentonite 33%, vermiculite 33% and humokarbowite 12%. F-M food value was determined according to the methods enforced [1]. Fatty acids composition in fish oil (herring-sprat) was examined using chromatographic method [14] – gas chromatograph coupled with mass spectrophotometer (GC/MS-mass spectrophotometer by Varian, Saturn 200). Purified and neutralized extracts were subjected to analysis according to

GC/MS technology with the se of capillary column Rt x MS of 30 m length. Fatty acid composition in fish oil was shown in other work [9].

Blood from examined cows was drawn from *vena exsterna jugularis*, at the first research day, after 2 weeks of preparation applying and in 4th and 8th experiment week. In blood serum there was determined total cholesterol level by enzymatic method as well as level of its fractions (HDL and LDL) by indirect method and using reagents of "Randox" firm house

The values obtained were statistically worked out using statistical program Statgraphics Version 5.0 and difference significance was estimated according to Duncan test.

RESULTS AND DISSCUSION

On the grounds of obtained results (Tab. 3) constant increase of total cholesterol level by all of examined groups was stated. During the whole period of experiment, the highest growth of cholesterol concentration in blood serum of examined cows was noted in the group of primiparous cows and multiparous, which didn't get the fish oil supplement to the fodder. This increase has amounted to adequately: 130% and 89% ($p \leq 0,01$) in relation to the level at the first day of experiment. In groups III and IV of experimental animals the increase was on the lowest level. By primiparous cows of III group it amounted to only 33% ($p \leq 0,05$). Loor et al. [11] have also noted in their studies an increase of cholesterol level in blood serum of cows of about 30%, after applying to the rumen 1% of fish oil in food ration. Such results confirm also other studies [4, 13]. Petit at all [13] applying cows 1,2% of fish oil, have noted increase of total cholesterol level in blood serum of examined cows of 36% in relation to the groups getting commercial preparation containing protected fat, what wasn't noted in own studies, where in the groups of control cows cholesterol concentration has risen more than by cows getting fish oil supplement to the food ration.

Table 3. Middle values of cholesterol level and its fraction in blood serum of examined cows

GROUPS	Samplings		Cholesterol [mmol/L]	HDL [mmol/L]	LDL [mmol/L]
I	Start	$\bar{x}$	**aA2,09**	**aA1,74**	**A0,32**
		SD	0,51	0,48	0,15
	2nd week	$\bar{x}$	**b3,02**	**bcA2,64**	**A0,27**
		SD	0,85	0,80	0,12
	4th week	$\bar{x}$	**aB3,61**	**aC2,99**	**a0,39**
		SD	0,84	0,76	0,17
	9th week	$\bar{x}$	**bB4,80**	**cBC4,03**	**Bb0,69**
		SD	1,17	0,96	0,37
II	Start	$\bar{x}$	**aA3,03**	**aA2,74**	**AC0,21**
		SD	0,89	0,84	0,10
	2nd week	$\bar{x}$	**bA4,02**	**b3,74**	**aC0,27**
		SD	0,88	0,90	0,08
	4th week	$\bar{x}$	**B5,35**	**B4,44**	**bBC0,59**
		SD	0,91	0,82	0,24
	9th week	$\bar{x}$	**B5,75**	**aB4,69**	**aB0,89**
		SD	1,18	1,10	0,43

Table 3. Continuation

GROUPS	Samplings		Cholesterol [mmol/L]	HDL [mmol/L]	LDL [mmol/L]
III	Start	$\bar{x}$	**a3,54**	**a2,07**	**A0,87**
		SD	1,32	0,54	0,58
	2nd week	$\bar{x}$	**a3,62**	**2,36**	**a1,21**
		SD	0,56	0,39	0,63
	4th week	$\bar{x}$	**4,19**	**b2,56**	**1,59**
		SD	0,89	0,46	0,71
	9th week	$\bar{x}$	**b4,74**	**2,47**	**bB 2,21**
		SD	0,99	0,36	0,97
IV	Start	$\bar{x}$	**A3,95**	**A2,10**	**AC1,28**
		SD	0,83	0,65	0,92
	2nd week	$\bar{x}$	**a4,69**	**aB2,75**	**C1,85**
		SD	0,62	0,70	0,47
	4th week	$\bar{x}$	**bB5,63**	**B2,90**	**aBC2,67**
		SD	0,83	0,52	0,75
	9th week	$\bar{x}$	**bB5,92**	**bA2,26**	**bB3,59**
		SD	1,29	0,41	1,33

Significance of differences between samplings in the given group at $p \leq 0.01$ – A, B, by $p \leq 0.05$ – a, b.

Result of transformations in total cholesterol level in blood serum of examined cows was also changes in the level of cholesterol fractions HDL and LDL. The greatest cholesterol concentration increase of HDL fraction, during the whole period of experiment was noted by cows of the control groups: I of 131% ($p \leq 0,01$) and II of 71% ($p \leq 0,01$). By animals getting in the food ration 1% fish oil supplement the increase was lower. In group of primiparous cows the growth amounted to 19% in the period of 8 weeks and wasn't statistically confirmed whereas in the IV group, after 2 weeks of applying fish fat there was noted growth of 38% ($p \leq 0,01$) and during next 6 weeks decrease of its concentration, what caused, that all the experiment long its increase was statistically unimportant and amounted to 7%. That tendency is confirmed by the studies of Petit at all [13], but not on such low level as in own studies. These authors have noted, after applying of fish oil supplement, an increase of fraction HDL concentration of 36% in relation to the cows getting protected fat in form of commercial preparation.

Identical tendency was observed in case of transformations by the cholesterol level of fraction LDL. By primiparous cows from control and experimental groups during 8 weeks of experiment there was observed cholesterol concentration increase of this fraction on similar level. In both cases it amounted to over 100% ($p \leq 0,01$). It's important to mention here, that by multiparous cows the changes were more distinct. By cows from IV group, getting fish oil supplement to the daily feed ration, cholesterol level increase of LDL fraction of 180% ($p \leq 0,01$) all the experiment long was noted. In analogous control group the increase amounted already 323% ($p \leq 0,01$). Such high rise wasn't noted by Petit at all [13] in their studies. After applying of fish oil in amount of 1,5% food ration they have noted an increase of cholesterol LDL fraction of 25% in relation to standard ration.

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INFLUENCE OF FISH OIL LONG-TERM ADDITION ON FATTY ACIDS CONTENT IN MILK OF DAIRY COWS

Kupczyński, R., Janeczek, W., Szołtysik, M.*, Pogoda-Sewerniak, K. and Korczyński, M.

*Wroclaw University of Environmental and Life Sciences, Department of Animal Hygiene and Environment, ul. Chełmonskiego 38c, 51–630 Wroclaw, Poland, * Department of Animal Products Technology, Wroclaw University of Environmental and Life Sciences, Poland*

SUMMARY

The aim of investigation was to evaluate of long-term addition of fish oil effect on fatty acids contents in milk fat. The cows were administered preparation containing fish oil (1% in DM) which resulted in decreased level of short chain fatty acids in milk fat and increased level of long chain fatty acid. The addition of mineral-fat preparation had a significant effect on growth of *cis*-9, *trans*-11 CLA content as well as transvaccenic acid and omega-3 fatty acids (EPA, DHA) in milk fat of cows. The growth of CLA was 364.8% in primiparous milk and 454.8% in multiparous milk, respectively.

Keywords: dairy cows, fish oil, fatty acids, conjugated linoleic acid, transvaccenic acid

INTRODUCTION

Milk fat contains several compounds with specific biological properties. One of these is conjugated linoleic acid (CLA). In animal models, CLA has been shown to inhibit the growth of cancer, exhibit anti-atherogenic effects, and alter body and bone metabolism [10, 15, 17]. Based in part on these observations, CLA is perceived to be a dietary constituent that may have potential benefit as a modulator of human disease.

CLA content in milk fat is determined by such factors as, e.g. breed, age, stage of lactation. The strongest effects belong to food factors [1, 5, 7, 12, 13, 14, 20]. Supplementation of food rations for cows with mono- and poly- unsaturated fatty acids in the form of seeds, extruded crushed meal, calcium oils and salts influences PUFA biohydrogenation in rumen content, modifying milk composition [2,3, 5, 7]. Linoleic and linolenic fatty acids supplied with forage undergo biohydrogenation in rumen due to *Butyrivibrio fibrisolvents* and other bacteria to stearic acid. In those alterations *cis*-9,*trans*-11 CLA isomer and vaccenic acid (VA, *trans*-11 $C_{18:1}$) are intermediate products [5, 14]. *Cis*-9,*trans*-11 CLA present in milk fat is synthesized in 64-78% in milk gland with VA by Δ^9-desaturase (stearoyl-CoA desaturase) [5, 8, 16]. *Cis*-9, *trans*-11 CLA synthesis in milk gland can range up to 91% of total CLA milk fat content [12]. Increasing VA is therefore an important part of this research.

Feeding lipid sources rich in mono- and polyunsaturated fatty acids, either as seeds, free oil, or calcium salts, increased the *cis*-9, *trans*-11 CLA and VA content of milk when oil is accessible to the rumen microorganisms for biohydrogenation. In previous studies [2, 18, 20] fat supplements were fed for not more than 4 wk. AbuGhazaleh et al. [1] used the simultaneous supplementation of 0.5% fish oil from fish meal and 2% soybean oil from extruded soybeans. Relatively high

growth of cis-9, trans-11 CLA in milk was apparent through first 5 weeks (in 3 weeks was 350%), and next content of these isomers slightly decreased.

The aim of this work was estimation of effect of fish oil long-term addition (on mineral support) in feeding of high productive dairy cows onto fatty acids content, with specially regard of *cis*-9, *trans*-11 CLA and n-3 polyunsaturated fatty acids (PUFA n-3).

MATERIAL AND METHODS

The experiment was executed in milk cows' farm with stock of 280 cows and with mean yield of 8200 kg of milk yearly. Experimental cows were kept in the same environmental conditions and they were fed of TMR (total mixed rations). The fodders used for cow feeding (TMR components) were subjected to chemical analysis in Blattin Laboratory in Langenfeld to determine the content of dry mass, total protein, raw ash, raw fat, raw fibre and its fractions (ADF and NDF), as well as mineral components: calcium, phosphorus, magnesium and sodium. On the basis of the analysis done, there were worked out food rations for cows according to DLG norms. Quantitative composition and food value of the doses were shown in Table 1. There were 40 cows in these studies, which were divided onto subsequent groups (n=10):

group I – control group (primiparous),
group II –primiparous, fed with 1% of fish oil in DM,
group III –control group (multiparous in 2 or 3 lactation),
group IV – multiparous (in 2 or 3 lactation), fed with 1% of fish oil in DM.

The fish oil was mixed with mineral component, additionally administered for 8 weeks twice a day in the dose of 563 g per head (1126 per day). Obtained mineral-fat preparation contained: fish oil (herring-sprat) – 25%, baidelit – 33%, wernikulit – 33% and humokarbowit – 9%. The preparation was added beginning from 1 week of lactation during 8 subsequent weeks. Composition of fatty acids from fish oil was marked by chromatographic method. The results of these analyses have been showed in other research [11].

The representative milk samples were taken from cows in day of beginning of research (1 week of lactation) and in day of ending up of research (after 8 weeks of FO addition). Fatty acid composition was determined in representative samples following gas chromatography method with the use of gas chromatograph Agilent Technologies 5973. Separation was carried out in the following conditions: column 60m x 0.25μm, column temperature ranged 140 °C (5 min) to 240 °C (4 °C/min), carrier gas – helium (20 m/s), spray 1 μl, 260 °C, split 100:1.

The values obtained were statistically worked out using statistical program Statgraphics Version 5.0 and difference significance was estimated according to Duncan test.

RESULTS AND DISSCUSION

The assessment of fatty acid contents in fish oil showed that it features high percentage of polyunsaturated fatty acids (PUFA) – 40.43% including linoleic acid (LA) 7.28%, eicosapentaenoic (EPA) 8.19% and docoshexaenoic acids (DHA) 13.83% [11]. In Poland the fish oils are obtained mainly from herrings, sprats a mackerels. American investigation involving cow food made use of fish oil originating from menhadan. That oil contained less EPA and DHA – 10.93 and 11.92 g / 100 g fatty acids respectively than fish oil applied in our own investigation

[2,3], while the content of polyunsaturated fatty acids was lower than the one in our own investigation.

Using of fatty-mineral preparation, which contained the fish oil (1% of DM) as an addition to TMR dose for cows caused decreasing of content of short-chain fatty acids in milk (C 4:0 – C12:0) and growth of long-chain content (C 16:1 – C22:6).

In day of beginning the study, concentration of *cis*-9, *trans*-11 CLA isomers were on similar level in individual groups (0.54 g/100g of fatty acids in groups I and III; and 0.64 g/100g of fatty acids II and IV). In day of the investigations finish, content of cis-9, trans-11 CLA were 1.97 (primiparous, group II) and 2.82 g/100g of fatty acids (multiparous, group IV). The growth was 364.8% in primiparous milk and 454.8% in multiparous milk, respectively. The significant growth of vaccenic acid concentration in milk, 387.8% (group II) and 255,9% (group IV) was found, respectively to 3.8 and 3.71 g/100g of fatty acids (tab. 2).

Although dietary fish oil dramatically and consistently increases milk VA and CLA concentrations, it can also decrease feed intake, milk production, and milk fat yield or concentration [6, 9, 20]. Donovan et al. [9] fed fish oil to dairy cows at 0, 1, 2, or 3% of diet DM and observed maximum concentrations of milk VA and CLA at 2% fish oil supplementation. Because fish oil contains low amounts of known precursors of VA and CLA, the authors speculated that fish oil enhanced the conversion of linoleic acid or linolenic acid, or both, from other feed sources into VA and CLA, possibly by inhibiting the final step in the biohydrogenation of VA to stearic acid [18]. Whitlock et al. [20] found that milk VA and CLA were increased similarly for cows fed 1% fish oil in combination with 1% fat from extruded soybeans compared with when cows were fed 2% fish oil. When cows were fed a diet containing 2% fat from extruded soybeans, VA and CLA concentrations increased less than half as much as when they were fed the fish oil-supplemented diets.

When fish oil and sunflower seeds (linoleic acid source) were fed, milk concentrations of *cis*-9, *trans*-11 CLA and VA averaged 1.7 and 3.74 g/100 g of fatty acids, respectively [2]. Milk fat from cows fed a control diet (no added fat) contains approximately 0.4 and 0.75 g/100 g fatty acids of *cis*-9, *trans*-11 CLA and VA [3]; therefore, feeding a blend of fish oil and unsaturated fat source such as extruded soybeans or sunflower seeds increased milk *cis*-9, *trans*-11 CLA and VA by 300 to 400%, respectively. Milk fat *cis*-9, *trans*-11 CLA and VA concentration (g/100 of fatty acids) and yield (g/d) were 2.5-fold greater for cows fed the fish meal and extruded soybeans diet over the 10 wk of fat supplementation [1]. In own investigation used mineral-fat preparation caused the growth of CLA In milk until to 450%. This kind connection of fish oil at long-term supplementation she was (8 weeks) very effective. Shingfield et al. [19] reported that administration of fish oil with sunflower oil resulted (on the fifth day) in higher *cis*-9, *trans*-11 CLA concentration up to 5.37g/100g fatty acids, while after 15 days of using the mentioned supplementation, concentration of *cis*-9, *trans*-11 CLA decreased to 2.35g/100g fatty acids.

Some authors particularly show that in TMR cow feeding system CLA value in milk remains lower in comparison to the milk collected from the cows fed on a pasture [6, 5, 12]. Therefore, our own investigation confirms purposefulness of the described supplementation of food rations, especially in the situation of common use of TMR system on highly efficient cow farms.

The fish oil, added to the diet caused growth of omega-3-polyunsaturated acids (EPA and DHA) content in milk, and it was higher in milk of multiparous cows. Mineral-fat preparation caused the growth of DHA after 8 weeks supplementation, to 0.18 and 0.31 g/100g of fatty acids in groups II and IV, respectively. That fact was also confirmed by other investigations [2, 3, 20] which proved that EPA and DHA concentration in cow milk fat did considerably increase after application of vegetable oils with fish oil. Generally, EPA and DHA transfer from food ration to

milk is low [1] since they are preferentially deposited in body tissues rather than in milk fat. The results obtained confirm purposefulness of fish oil supplementation in cow food rations which is also reflected in milk nutritional value in human diet.

CONCLUSIONS

The addition of mineral-fat preparation had a significant effect on growth of *cis*-9, *trans*-11 CLA content as well as transvaccenic acid and omega-3 fatty acids (EPA, DHA) in milk fat of cows. The growth of CLA was 364.8% in primiparous milk and 454.8% in multiparous milk, respectively.

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Table 1. Content and nutritional composition of the cows' diets

Dose composition	Units		
Maize silage	kg	25,000	
Fresh pressed pulp		8,000	
Maize seed silage		5,500	
Barley		3,000	
Soybean		2,700	
Rapeseed		2,500	
Barley strain		2,000	
Sodium bicarbonate		0,200	
Premix		0,180	
Forage chalk		0,150	
Fat-mineral preparation*		1,125	
Nutritional composition		Control groups I and III – (primiparous and multiparous)	Experimental groups II and IV (primiparous and multiparous)
Dry mass	%	48,17	49,06
Raw fibre % dry mass	% s.m.	14,03	13,65
NEL	MJ/kg s.m.	6,87	8,24
Total protein	g/kg s.m.	3858,00	3864,59
Available total protein in small intensive	g/kg s.m.	3806,63	3813,19
Ca	g/kg s.m.	6,95	6,75
P	g/kg s.m.	4,36	4,35
Na	g/kg s.m.	1,40	1,35
Mg	g/kg s.m.	2,54	3,19

*preparation supplementation (containing fish oil) in experimental groups (II and IV)

Table 2. Fatty acids composition in milk fat of cows (g/100g fatty acids)

Fatty acids	Group I – control		Group II – experimental		Group III – control		Group IV – experimental	
	Period of T-M preparation administration							
	0	8th week	0	8th week	0	8th week	0	8th week
$C_{4:0}$	2,89	2,72	2,89	2,54	3,14 ^{A}	3,25 ^{A}	3,14 ^{A}	1,99^{B}
$C_{6:0}$	1,25^{A}	1,53^{B}	1,25 ^{A}	1,00 ^{B}	2,34 ^{A}	2,31 ^{B}	2,34 ^{A}	1,82 ^{B}
$C_{8:0}$	1,73	1,62	1,73 ^{A}	0,93 ^{B}	1,82 ^{A}	1,73 ^{A}	1,82 ^{A}	1,31 ^{B}
$C_{10:0}$	2,36	2,33	2,36 ^{A}	1,82 ^{B}	2,84 ^{A}	2,6 2 ^{B}	2,84 ^{A}	2,01 ^{B}
$C_{12:0}$	3,01 ^{A}	3,37 ^{B}	3,01 ^{A}	3,11 ^{B}	3,56	3,41	3,56	3,54
$C_{14:0}$	11,83 ^{A}	10,52 ^{B}	11,83 ^{A}	9,97 ^{B}	11,93 ^{A}	10,58 ^{B}	11,93 ^{A}	10,21 ^{B}
$C_{14:1}$	0,56 ^{A}	0,51 ^{A}	0,56 ^{A}	0,44 ^{B}	0,62 ^{A}	0,59 ^{B}	0,62 ^{A}	0,47 ^{B}
$C_{16:0}$	29,13 ^{A}	30,12 ^{B}	29,13 ^{A}	30,62 ^{B}	30,24	30,50	30,24	31,02
$C_{16:1}$	1,02	1,01	1,02 ^{A}	2,28 ^{B}	1,24 ^{A}	1,04 ^{B}	1,24 ^{A}	2,99 ^{B}
$C_{18:0}$	11,40 ^{A}	12,41 ^{B}	11,40 ^{A}	10,22 ^{B}	12,03	12,56	12,03 ^{A}	11,03 ^{B}
$C_{18:1t9}$	0,14	0,15	0,14 ^{A}	0,25 ^{B}	0,23	0,20	0,23 ^{A}	0,34 ^{B}
$C_{18:1c9}$	18,36	18,8	18,36	16,83	17,45	18,36	17,45	16,01
$C_{18:1t11\ (TVA)}$	0,98^{A}	0,99 ^{A}	0,98 ^{A}	3,80^{B}	1,45 ^{A}	1,12 ^{A}	1,45 ^{A}	3,71^{B}
$C_{18:2t9,t12}$	0,21 ^{A}	0,23	0,21 ^{A}	0,33^{B}	0,17 ^{A}	0,15 ^{A}	0,17 ^{A}	0,40^{B}
$C_{18:2c9,c12}$	2,78 ^{A}	2,79 ^{A}	2,78 ^{A}	1,63^{B}	3,11 ^{A}	3,09 ^{A}	3,11 ^{A}	1,89^{B}
$C_{18:2c9,t11(CLA)}$	0,54^{A}	0,63^{B}	0,54^{A}	1,97^{C}	0,62^{A}	0,60^{A}	0,62^{A}	2,82^{B}
$C_{18:2t9,t11(CLA)}$	0,02	0,03	0,02	0,08	0,05^{A}	0,06^{A}	0,05^{A}	0,18^{B}
$C_{18:3\ n-3}$	0,04	0,05	0.04	0,06	0,03	0,09	0.03 ^{A}	0,20^{B}
$C_{18:3\ n-6}$	0,16	0,18	0,16	0,61	0,17	0,22	0,17	0,80
$C_{20:1}$	0,08	0,10	0,08	2,42	0,07	0,15	0,07	2,95
$C_{20:4\ n-6}$	0,16	0,16	0,16	0,27	0,18	0,19	0,18	0,31
$C_{20:5\ n-3\ (EPA)}$	0.03^{A}	0,03^{A}	0.03^{A}	0,40^{B}	0.02^{A}	0,02 ^{A}	0.02 ^{A}	0,46^{B}
$C_{22:6\ n-3\ (DHA)}$	–	–	–	0,18	–	–	–	0,31
Σ	85,46	90,28	88,68	91,57	93,31	90,02	93,31	96,77
Other	14,54	9,72	11,32	8,43	6,69	9,98	6,69	3,23
Short-chain[1]	11,24	11,57	11,24	9,4	13,7	10,07	13,7	10,67
Medium-chain [2]	42,54	42,16	42,54	43,31	44,03	42,71	44,03	44,69
Long-chain [3]	34,76	36,55	34,9	39,05	35,58	36,61	35,58	41,41
Saturated	65,18	64,62	65,18	60,21	69,76	65,97	69,76	62,93
Unsaturated	24,94	25,66	25,08	31,55	25,41	24,05	25,41	33,84
∑ EPA, DHA	0,03	0,03	0,03	0,58	0,02	0,02	0,02	0,77
CLA*/ TVA	0,55	0,64	0,55	0,52	0,43	0,54	0,43	0,76
n-6/ n-3	4,57	4,25	4,57	1,38	7,00	3,73	7,00	1,14

TVA – vaccenic acid, CLA – conjugated linolic acid, EPA – eicosapentaenoic acid, DHA – docohexaenoic acid

[1] – short-chain fatty acids (C4:0 – C12:0); [2] – medium-chain fatty acids (C14:0 – C16:1); [3] – ling-chain fatty acids (> C16:0)

* $C_{18:2c9,t11}$ CLA

A,B – significant differences ($p<0.01$)

EFFECTS OF MANNAN OLIGOSACCHARIDES IN THE DIET OF BEEF CATTLE IN THE TRANSITION PERIOD

Tassinari, M.[1], Pastò, L.F.[1], Sardi, L.[1] and Andrieu, S.[2]

[1] *Dipartimento Morfofisiologia Veterinaria e Produzioni Animali, Università di Bologna, Italy;*
[2] *Alltech Biotechnology Centre, Dunboyne, Ireland*

SUMMARY

Forty-eight male Blond d'Aquitaine calves were used to study the effects of mannan oligosaccharide (MOS) during the first phase of the cattle fattening cycle (48 days). Calves were divided into two treatment diets: Control (straw and pellet feed) and MOS (Control diet plus 0.4% in pellet feed of BIO-MOS®, Alltech, Inc.). The daily weight gain was higher (+3,6%) in MOS group than in Control group. Alpha globulins, beta globulins and NEFA were significantly higher in Control than in MOS group. The calves treated with MOS showed lower levels of stress and showed a better immune system response.

Keywords: mannan oligosaccharide, young bulls, performance, blood proteins, IGF1, NEFA

INTRODUCTION

Since 1st January 2006, it is no longer possible to use auxinic antibiotics in livestock feed in the European Community. In beef cattle alternatives to these molecules have been studied for several years, particularly substances such as prebiotics or parabiotics, "natural" substances which do not constitute any risk to animals or human (so-called additives without residues). Oligosaccharides are promising alternatives to antibiotic growth promoters because they facilitate and support the symbiotic relationship between host and microflora. Fructooligosaccharides (FOS) and Mannan oligosaccharides (MOS) are two classes of oligosaccharides that are beneficial to enteric health, but they differ on their mode of actions. FOS influence enteric microflora by "feeding the beneficial bacteria", which competitively excludes the colonization of pathogens (Mordenti and Panciroli, 1995; Ferket, 2004). Unlike FOS, MOS is not used as a substrate in microbial fermentation, but still exerts a significant growth-promoting effect by enhancing the animal's resistance to enteric pathogens (Ferket, 2004). MOS is a glucomann protein complex derived from *Saccharomyces cerevisiae*. Yeast cells are lysed, and the resulting culture is centrifuged to isolate the cell wall components, which are subsequently washed and spray dried (Spring et al., 2000). MOS have been used with interesting results in various livestock species, demonstrating an improvement in performance, better immune system function and a reduction in intestinal pathogens.

Bio-Mos® (Alltech, Inc., Nicholasville, KY) is the commercial source of MOS that has been used in most of the published research literature. Based on the scientific literature, Bio-Mos® enhances resistance to enteric disease and supports growth performances by the following means: 1) inhibits colonization of enteric pathogens by blocking bacterial adhesion to gut lining; 2) enhances immunity; 3) modifies microflora fermentation to favour nutrient availability for the host; 4) enhances the brush border mucin barrier; 5) reduces enterocyte turnover rate; and

6) enhances the integrity of the gut lining (Newman, 1994; Savage et al., 1996; Spring et al., 2000; Ferket, 2004).

In chickens, immunoglobulins (Ig) concentrations were greater in birds receiving MOS compared with birds receiving a control diet (Savage et al., 1996). Supplementation of broiler chicks with MOS beneficially influenced the bacterial populations in the digestive system (Spring et al., 2000). Several studies in pigs have reported improvements in various measures of performance or immune function such as gain, feed conversion, lymphocyte transformation, and Ig concentrations, compared with unsupplemented animals (Newman and Newman, 2001; O'Quinn et al., 2001; Davis et al., 2002; White et al., 2002). Research with cattle regarding the effects of MOS supplementation has been primarily in calves and heifers and has concentrated on alteration of the intestinal microflora. In calves fed a commercial milk replacer, MOS supplementation resulted in lower faecal coliform concentrations and decreased respiratory disease incidence than non supplemented calves (Newman et al., 1993). Other field data suggest that antibiotics in milk replacers can be replaced with mannan oligosaccharides to obtain similar calf performance (Heinrichs et al., 2003). The supplementation of MOS to cows during the dry period enhanced their immune response to rotavirus and tended to enhance the subsequent transfer of rotavirus antibodies to calves (Franklin et al., 2005).

The objective of this research was to study the effects of MOS on beef cattle, in particular in calves returning to the shed in the first phase of the cattle fattening period (the transition phase).

MATERIALS AND METHODS

Forty-eight male Blond d'Aquitaine calves, just arrived in Italy together from France, by lorry in a journey lasting 8 hours, were weighted and homogeneously divided into 8 boxes with straw bedding (6 calves per box), and assigned to two experimental groups. Control Group: 24 calves, fed with commercial cattle feed (starting feed) and hay (in the first 7 days) and straw; MOS Group: 24 calves, fed in the same way as the Control Group with the exception of the addition to the commercial cattle feed of Bio-Mos® (Alltech, Inc., Nicholasville, KY) at a ratio of 4g per kg of feed (0.4%). The commercial feed (products and by-products of cereals in grains; products and by-products of oily seeds; dried forage; products and by-products of the production of sugar; minerals) was formulated to provide 14% Crude Protein, 4% Fat, 11% Fibre and 7% ash on fresh product basis. In the first 48 hours after the arrival, calves were given access to *ad libitum* hay only which is a normal practice at farm level. Feed and straw were gradually introduced on the third day and until the seventh day.

The sanitary condition of the calves was checked every day. The consumption of feed offered to the calves in each box was evaluated daily. The research lasted 48 days and during this period blood samples were obtained from 24 calves (12 per group). Blood samples were collected in vacutainer tubes without anticoagulant via jugular venipuncture on days 0, 22 and 48 and analysed for serum protein concentrations (albumin, alpha, beta and gamma globulin), IGF1 and NEFA. On the last day of the test and at the time of the last blood sample the calves were re-weighted in order to calculate the overall live weight gain and the average daily weight gain. On their arrival day (the first day of the experiment) all the calves were vaccinated against respiratory illnesses (IBR, Syncitial Virus and Pasteurella), against BVD and treated with Ivomec® (against intestinal parasites). After about 20 days a second vaccination took place (booster).

All the data obtained were subjected to mathematical-statistical elaboration, using analysis of the variables (SAS System, 1996).

RESULTS AND DISCUSSION

During the research all the calves ate regularly both feed and straw. In Table 1, performance of the calves during the course of the research is shown. As can be observed the average live weight at the beginning of the trial was almost similar for both treatments (225.83 kg *vs* 226.25 kg, respectively for Control and MOS Group). The final live weight, at 48 days of trial, was 281.665 kg and 284.09 kg, respectively for Control and MOS Group. The total average live weight gain for Control Group was 55.835 kg, while the MOS Group had an average increase of 57.840 kg. Therefore, calves in MOS Group showed a greater daily weight gain (DWG) than those in the Control Group (1.205 kg *vs* 1.163 kg per head per day). The biggest increase in weight in the MOS Group (+ 3.6%), however, cannot be explained by a greater consumption of feedstuffs. Indeed, the average daily feed consumption (shown in Table 1), for the two groups was similar (3.968 kg *vs* 3.990 kg per head per day, respectively for Control and MOS Group). Regarding health, animals experienced only the classic problems occurring at the return of cattle to the shed (respiratory and intestinal diseases above all due to the stress of the transport and mixing of animals from various origins). The better daily weight gain of the calves of MOS Group may be attributed to the presence in the feed of the Mannan Oligosaccharides, as the number of animals treated by drugs during the research period was the same in each Group (16 calves).

Table 1. Performance of calves during the research

		CONTROL	MOS
Calves	n	24	24
Initial weight	kg	225.83	226.25
Final weight	kg	281.665	284.09
Total Weight gain	kg	55.835	57.840
DWG	kg	1.163	1.205
Daily Feed intake (concentrate)	kg/d	3.968	3.990

As far as the blood proteins are concerned (Table 2), it should be underlined that while the albumin values are the same (48.71% *vs* 48.84%, respectively for Control and MOS Group), an interesting response by the calves to the globulins is observed. A significant difference (P=0.017) in the alpha globulins in the 3rd blood sample is found, corresponding to the 48th day of the research (17.65% *vs* 16.34%, respectively for Control and MOS Group) and for the beta globulins (P=0.018) as a medium value during the course of the research (11.57% *vs* 10.81%, respectively for Control and MOS Group). The gamma globulins showed a tendency towards higher values in the MOS Group as compared to the Control Group (25.68% *vs* 27.41% on the 48th day, and average values of 22.28% *vs* 23.25%, respectively in Control and MOS Groups). Whilst not being significant differences in this case, this result has practical meanings as higher figures for alpha and beta globulins in the Control Group are a general indicator for the organism's response to inflammatory factors; the higher quantities of gamma globulins, on the other hand, can be linked to a better response of the calves to the vaccination (and subsequent booster). Overall, therefore, it can be concluded that the Mannan Oligosaccharides in Bio-Mos® contributed to the better immune system response of the treated calves. This is confirmed by NEFA results (Table 2): the lower values found in the MOS Group in the blood tests on the 48th day of research (124.58uEq/l *vs* 195.42 uEq/l), demonstrate that the mobilization of the fatty acids was significantly lower (P=0.046). This indicates as well that the animals showed lower levels of stress or, more simply,

that the calves have responded better, thanks to the Mannan Oligosaccharides to the various elements of stress in this delicate phase in the fattening process. Recently re-introduced to the shed and just arrived from France, calves are indeed exposed to various stress factors (starting with the removal from pasture, mixing with other calves, transport in lorries, fasting which can last 48–72 hours, formation of new groups in new places, etc.) and the response to such factors is now well known. The evaluation of the NEFA, therefore, is a useful index of the stress of the animal. The significantly lower values in the calves given MOS indicate a better response of these animals to the situation and it is confirmed by the greater increase in weight in this period.

Table 2. Blood protein, IGF1 and NEFA values of calves from Control and MOS Group

		CONTROL (n = 12)	**MOS (n = 12)**	P value
Blood proteins				
Albumin				
Day 0	%	53.85 ± 2.05	53.48 ± 1.85	0.642
Day 22	%	47.02 ± 2.02	47.66 ± 2.16	0.465
Day 48	%	45.25 ± 4.23	45.40 ± 3.28	0.923
Average 0–48 days	%	48.71 ± 4.73	48.84 ± 4.22	0.898
Alpha				
Day 0	%	16.12 ± 1.08	16.65 ± 1.23	0.270
Day 22	%	18.53 ± 0.95	18.30 ± 1.17	0.597
Day 48	%	17.65 ± 1.43	16.34 ± 1.02	0.017
Average 0–48 days	%	17.43 ± 1.52	17.10 ± 1.41	0.335
Beta				
Day 0	%	12.35 ± 0.95	11.42 ± 1.67	0.109
Day 22	%	10.95 ± 1.05	10.14 ± 1.08	0.077
Day 48	%	11.42 ± 1.66	10.85 ± 0.90	0.302
Average 0–48 days	%	11.57 ± 1.36	10.81 ± 1.34	0.018
Gamma				
Day 0	%	17.68 ± 2.79	18.45 ± 3.22	0.539
Day 22	%	23.49 ± 2.67	23.90 ± 2.64	0.709
Day 48	%	25.67 ± 5.15	27.41 ± 3.57	0.348
Average 0–48 days	%	22.28 ± 4.97	23.25 ± 4.84	0.405
Total Proteins				
Day 0	g/l	65.58 ± 3.06	64.25 ± 4.69	0.418
Day 22	g/l	60.42 ± 2.31	63.08 ± 10.72	0.408
Day 48	g/l	64.33 ± 7.10	65.25 ± 5.08	0.719
Average 0–48 days	g/l	63.44 ± 5.05	64.19 ± 7.21	0.610
IGF1				
Day 0	ng/ml	5.44 ± 4.04	5.83 ± 5.90	0.851
Day 22	ng/ml	12.77 ± 8.57	14.37 ± 11.30	0.699
Day 48	ng/ml	24.63 ± 13.77	16.49 ± 13.70	0.160
Average 0–48 days	ng/ml	14.28 ± 12.34	12.23 ± 11.48	0.468
NEFA				
Day 0	uEq/l	494.98 ± 195.71	552.39 ± 244.68	0.532
Day 22	uEq/l	125.79 ± 49.90	107.29 ± 33.55	0.298
Day 48	uEq/l	195.42 ± 96.58	124.58 ± 64.58	0.046
Average 0–48	uEq/l	272.07 ± 205.28	261.42 ± 253.13	0.845

Results on total proteins (Table 2) show no difference between the 2 groups (average 63.44 g/l *vs* 64.19 g/l, respectively for Control and MOS Group) but the tendency towards lower values for alpha and beta globulins and higher values for gamma globulins is definitively a positive result of this research.

The IGF1 values, also in Table 2, do not show significant differences, either during the course of the blood samples or as average. It is worth considering, however, that the figures show a high level of variation within the 2 groups, particularly in the last blood sample. The average values produced by the research are 14.28 ng/ml *vs* 12.23 ng/ml, respectively in the Control and MOS Groups.

CONCLUSIONS

Positive effects of MOS on sow reproductive performance were reported by Funderburke (2001): when added to the gestation and lactation diet in a mixed parity herd weaning at 21 days, an increase in birth weight, a decrease in pre-weaning mortality, increased pre-weaning growth rate and a quicker return to oestrus were noted; the study also showed a significant increase in the immunoglobulins levels in the colostrum. It has also been noted that MOS can modulate intestinal flora, significantly reducing numbers of harmful intestinal bacteria without damaging the lactobacillus and can also reduce diarrhoea problems in the weaning phase of the piglets (Spring et al., 2000; Newman and Newman, 2001; White et al., 2002). Positive effects have also been noted in relation to improved performance in turkeys, due, above all, to the capacity of MOS to strongly reduce Gram-negative pathogens (particularly *Enterococcus*) in the intestine (Savage et al., 1996). In newly born calves a tendency to improve the immune response to rotavirus has been noted by giving MOS to the mothers in the three weeks prior to the birth which is transferred through the colostrum (Franklin et al., 2005).

The results of this first research regarding the effects of Mannan Oligosaccharides (Bio-Mos®) on the performance of calves in the return to the shed phase have given interesting ideas for discussion.

Our results indicate a greater average daily weight gain (+ 3.6%) and a better response to stress for the calves of MOS Group compared with those of Control Group. The highest levels of gamma globulins (approximately 1 gram extra per litre of blood) found in the blood of calves which have been given MOS in their diets for 48 days, the lower levels of alpha and beta globulins and, above all, the significantly lower values of NEFA, indicate that the calves have responded better to the stress of this delicate phase.

These first results need further development, but the general trend indicated in this research leads us to state that there are positive aspects on the performance and metabolic response to stress in calves that are given Mannan Oligosaccharides (Bio-Mos®, Alltech, Inc., Nicholasville, KY) in their diets at a ratio of 4 g per kg of feed (0.4%) in the first 48 days after the return to the shed.

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POSTER PRESENTATIONS

CYTOTOXICITY OF THE MYCOTOXINS IN FEEDSTUFFS

Bakutis, B. and Baliukoniene, V.

Lithuanian Veterinary Academy, Department of Food Safety and Animal Hygiene, Tilzes 18, 47181 Kaunas, Lithuania

SUMMARY

Mycotoxins are secondary metabolites produced by filamentous fungi that cause a toxic response when ingested by higher animals. In colder more temperature regions: Canada, USA and most European countries as Lithuania fusariotoxins: DON, ZEN and ochratoxins are found more frequently. These mycotoxins may potentially affect animal health and productivity. DON exposure leads to apoptosis both in and in vivo in several organs such lymphoid organs haematopoietic tissues, liver and intestinal crypts intoxications. ZEN has high binding affinity for the intra-cellular oestrogen receptor and can enhance the proliferation of oestrogen responsive tumour cells. Ochratoxins (ochratoxin A) have an effect on immunoglobulins and phagocytic cells. The aim of this study was to investigate the cytotoxicity of the most commonly found mycotoxins: DON, ZEN, ochratoxins in naturally contaminated grains and feedstuffs samples.

Keywords: apoptosis, deoxynivalenol, feedstuffs, K-562, MH-22A, ochratoxins, SPEV, zearalenone

MATERIALS AND METHODS

K-562 human hematopoetic and swine kidney (SPEV) cell lines were cultured RPMI Medium 1640 w/glutamine, MH-22A mice-derived hepatoma cell line was cultured in DMEM supplemented with 10% FBS and antibiotics under standard conditions.

Naturally contaminated grains and feedstuffs were purified after extraction acetonitrile/water (84/16). Samples extracts were purified using the Mycosep # 227 cleanup column for DON, ZEN – Mycosep # 226, ochratoxins – MultiSep # 212. The clear extracts were evaporated Romer-Evap^{TM} system and the residues were dissolved in cell medium and incubated in triplicates as two-fold dilution series with cell lines on a 96-well microtiter plate for 24 h. Cells were seeded at a density of $1x10^{-3}$ – $1x10^{-4}$ cells/ml. The viability of cell population was examined using the crystal violet [6] and MTT assay [3]. Cells viability was assayed spectrophotometrically using a Multiskan MS photometer (Finland).

The DON, ZEN, aflatoxins concentrations were analyzed thin layer chromatography (TLC) method. Ochratoxins, T-2 toxn were analyzed by the enzyme-linked immunosorbent assay (ELISA). Veratox test kits (Neogen Corporation, USA) were used for the analysis. The cytotoxicity of DON, ZEN, ochratoxins was determined by measuring different endpoints such as inhibition of protein and DNA synthesis, plasma membrane integrity and reduced metabolic activity. The IC_{50} value (the concentration of each sample reducing the total response to a 50% value of untreated cells) for cytotoxic compound was calculated.

RESULTS AND DISCUSSION

DON cause necrosis and haemorrhage throughout the digestive tract, depress blood regenerative processes in the bone marrow and spleen. DON has been shown to be potent inhibitors of the eucaryote protein synthesis. Cellular effects on DNR synthesis, DNR breakage and membrane integrity have been considered to be secondary effects of the inhibited protein synthesis [4]. DON exposure leads to apoptosis both in vitro [7] and in vivo in several organs such lymphoid organs haematopoietic tissues, liver and intestinal crypts intoxications [1].

A finding which according to the authors indicated that inhibition of the protein synthesis and apoptosis are the main mechanisms for DON toxicity in the cells [5].

In this study we evaluated the cytotoxic effect of grain and feedstuffs contaminated with DON (table 1). Cells lines K-562 and MH-22A was used to evaluate the effects of DON on the blood cells.

Grains and feedstuffs contaminated with DON concentration 1000–3000 ng/ml have significant effect for cell line K-562. Feedstuffs with DON caused apoptosis K-562 cells 60±15%. There were no significant differences between toxicity assay grain and feedstuffs for K-562. Significant differences of were feedstuffs for MH-22A – 56–118%. The DON-free feedstuffs samples had a cytotoxic effect on the K-562 cells equivalent to DON. A recent study has shown that less than 10 µmol/ml DON (0,296 µg/ml) selectively inhibited some intestinal transport protein in human intestinal epithelial cells when the cells were incubated with the toxin for 24 and 48 hours.

Table 1. Cytotoxicity of deoxynivalenol and concentration of mycotoxins deoxynivalenol, T-2 toxin, zearalenone, ochratoxins, aflatoxins in grain and forages (500 mg extract/ml)

Samples	[a]Toxicity assay Cell cultures		[b]IC_{50} Cell cultures		Mycotoxins concentration (ng/ml)				
	K-562	MH-22A	K-562	MH-22A	DON	T-2	ZEN	OT	AFL
Triticale	58	90	1751±264	1158±257	2000	[c]nc	50	nc	1
Barley	53	109	472±57	460±30	1000	nc	130	nc	*d*
Wheat	67	118	1951±274	1173±157	3000	nc	500	nc	nc
Farrowing sows composite forage	53	73	1180±115	856±67	1250	nc	400	nc	*d*
Fattening pigs composite forage	68	56	1112±136	1339±339	1500	20	300	nc	1

[a] % apoptosis of cell cultures exposed to duplicate samples at 500 mg extract/ml compared to cells.
[b] IC_{50} (mg extract/ml cell culture medium). Data as mean ± standard deviation of at least three experiments.
[c] Not calculated.
[d] Not detected (concentration below the limit of detection).

Zearalenone is usually non-lethal to animals, but it is important to animal producers because its hyperestrogenic effects adversely influence the reproductive performance of animals. ZEN has high binding affinity for the intra-cellular estrogens receptor and can enhance the proliferation of oestrogen responsive tumour cells. There have been suggestions of the involvement of ZEN in human cervical cancer and premature initial breast development [2].

The cytotoxic effect of grain contaminated with ZEN was 394–563 ng/ml (table 2). Toxicity effect the grain samples were 46±6% for K-652, MH-22A – 52±23%. Feedstuffs with ZEN had

toxicity effect for MH-22A bigger. According to our data, K-562 cells and MH-22A proved to be resistant to grain samples contaminated with ZEN.

Table 2. Cytotoxicity of zearalenone and concentrations of mycotoxins deoxynivalenol, T-2 toxin, zearalenone, ochratoxins, aflatoxins in grain and forages (562 mg extract/ml)

Samples	[α]Toxicity assay Cell cultures		[b]IC_{50} Cell cultures		Mycotoxin concentration (ng/ml)				
	K-562	MH-22A	K-562	MH-22A	DON	T-2	ZEN	OT	AFL
Triticale	53	41	388±37	489±54	8,4	[c]nc	394	nc	0.4
Barley	37	46	782±143	634±148	8.4	nc	563	nc	0.4
Pease	47	69	627±162	424±93	0.56	nc	563	nc	*d*
Gestating sows composite forage	87	56	396±69	613±119	0.56	nc	675	nc	nc
Farrowing sows composite forage	99	73	285±191	387±109	0.56	nc	563	nc	nc

[α] % apoptosis of cell cultures exposed to duplicate samples at 500 mg extract/ml compared to cells.
[b] IC_{50} (mg extract/ml cell culture medium). Data as mean ± standard deviation of at least three experiments.
[c] Not calculated.
[d] Not detected (concentration below the limit of detection).

According to our data, K-562 cells and MH-22A proved to be resistant to grain samples contaminated with ZEN.

Ochratoxins (ochratoxin A) damages the kidneys of many types of animals. High concentrations of dietary ochratoxins can cause liver damage as well intestinal necrosis and haemorrhage. Ochratoxin A has been shown suppress immunity and to be carcinogenic. General indicators of immunosuppression following ochratoxin A ingestion include lymphocytopenia and depletion of lymphoid cells. This mycotoxin has an effect on immunoglobulins and phagocytic cells [1].

The cytotoxic effect of grain and feedstuffs contaminated with ochratoxins were 49±16% swine kidney SPEV cells, K-562 cells. Insignificant toxic effect was for MH-22A.

Table 3. Cytotoxicity of ochratoxins and concentrations of mycotoxins deoxynivalenol, T-2 toxin, zearalenone, ochratoxins, aflatoxins in grain and forages (875 mg extract/ml)

Samples	[α]Toxicity assay Cell cultures			[b]IC_{50} Cell cultures			Mycotoxin concentration (ng/ml)				
	SPEV	K-562	MH-22A	SPEV	K-562	MH-22A	DON	T-2	ZEN	OT	AFL
Barley	48	48	80	123±12	129±28	73±2	175	17	189	118	*d*
Barley	61	54	94	17±5	19±2.5	11±4	*d*	39	*d*	20	*d*
Wheat	45	55	89	39±1	32±3	20±3	*d*	[c]nc	*d*	35	nc
Wheat	48	51	101	22±5	20±3	10±0.5	nc	nc	nc	20	nc
Piglets composite forage	45	39	119	23±2	23±2	10±4	*d*	0.9	*d*	23	*d*

[α] % apoptosis of cell cultures exposed to duplicate samples at 500 mg extract/ml compared to cells.
[b] IC_{50} (mg extract/ml cell culture medium). Data as mean ± standard deviation of at least three experiments.
[c] Not calculated.
[d] Not detected (concentration below the limit of detection).

About 50% of the SPEV and K-265 cells were obviously dead after exposure ochratoxins concentrations 20–23 ng/ml in samples extracts.

The fact, that higher mycotoxins concentrations have toxic effect for different cell cultures. Despite of using different cell cultures and cytotoxicity endpoints than other authors, the our results comparable to literature data.

CONCLUSIONS

Cell culture systems can be more sensitive and more reproducible than tests involving intact animals. These cell culture assays can be used for the screening of toxicity of mycotoxins. The comparison of toxic responses obtained with each bioassay may orient to its toxicological mechanism.

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BACTERIAL PROBIOTIC ADDITIVE (*PEDIOCOCCUS ACIDILACTICI*) AND ITS IMPACT ON BROILER CHICKENS HEALTH AND PERFORMANCE

Chafai, S., Fatiha, I. and Alloui, N.

ESPA laboratory, Veterinary Department, Batna University, 05000; Algeria;
Email: ridan2002@hotmail.com

SUMMARY

Antibiotics were very important pieces of the puzzle that enabled the poultry production to move from a backyard flock based industry to the large-scale production facilities of today. Public health professionals have suggested that the use of subtherapeutic antibiotics in animal production may be partially responsible for the development of antibiotic resistant bacterial populations. The probiotics may be substituted by antibiotics (growth promoting) in certain cases. *Pediococcus acidilactici* is a bacterial probiotic used in this experience.16000 broiler chickens were assigned in two experimental groups: treatment (10^9 cfu/kg of feed of *Pediococcus acidilactici* MA18/5M) and control. In each group 8000 broiler chickens were allocated in the same batch and divided by a physical barrier. Individual live weight of a sample of 200 birds for each group from day 0 to day 56 was measured weekly. Feed intake, feed efficiency, mortality, carcass quality, serum lipids (cholesterol and triglycerides) and number of white blood cells, were recorded per group. The administration of *Pediococcus acidilactici* affected positively the growth performance of broilers (2586.43 vs 2252.79 g and feed conversion ratio (2.00 vs 2.5). There were no significant difference between groups in dressing, breast meat and thigh percent, at the end of day 56. Analysis of variance showed significant difference between treatments for serum lipids ($p \leq 0.01$). Mortality was almost similar in both groups (6.56 vs 6.51). The numbers of white blood cells were significantly affected by dietary treatment.

Keywords: *Pediococcus acidilactici*, broiler chickens, performance of production, health

INTRODUCTION

The development of resistance to certain antibiotics poses real problems to the animal and public health (Barton 2000, Hofacre *et al.,* 2001). Consequently, many additives (prebiotics, probiotics, symbiotics…) raise a particular interest as products of substitution to antibiotics in order to improve the production performances and the health of animals (Bach 2001, Revington 2002).

Pediococcus acidilactici is a probiotic bacterium that presents positive effects on the balance and the role of the intestinal flora, it also reinforces the immune defense and improves the production performances of animals (Jin *et al.,* 2000, Coppola and Turnes 2004, Stella 2005).

The objective of this study is to evaluate the effect of addition of *Pediococcus acidilactici* in the feed on the production performances (feed intake, weight gain, feed ratio and carcass yield), and on the blood lipids' concentration and the immunity of broiler chickens.

MATERIALS AND METHODES

1.1. Place of the study

The trial has been conducted at the Poultry Centre of Tazoult (Batna), Algeria. This centre is constituted of 10 buildings having the same technical features (materials of construction, surface area, extractors, pad colling, food and watering chains). Buildings having served to the experimentation have a surface area of 1000 m^2.

1.2. Animals

The trial has been conducted on 16 000 chicks of the strain ISA 15, coming from the same hatchery. They were allocated to two treatment groups of 8000 chicks each (control group and experimental group), raised separately in two identical buildings. Animals have been followed during all the trial period of 56 days of raising (from the 23/02 to 19/ 04/2005). At each weighing, 200 subjects were chosen randomly from both groups for individual weighing.

1.3. Feed

The feed is supplied by the centre of Tazoult that possesses its own unit of feed manufacture. Three types of feed have been distributed according to periods of raising: a starter feed (d0–d21), a grower feed (d22–d42) and a finisher feed (d43–d56). (Table 1)
Two treatments have been compared in this survey:

A control group (Cont.) receiving a classic feed based on maize and soyabean meal and an experimental group (Exp.) fed with the same feed than the (Cont.) combined with 10^9 ufc of Pediococcus acidilactici (MA 18/5M) /kg, equivalent to 100 grams of probiotic per ton of feed. Neither antibiotic, nor anticoccidial has been added to the feed.

1.4. Measured parameters

During the experimental period, feed intake, individual live weight of 200 birds per group, feed ratio and mortality rate have been measured weekly for both treatment groups.

At the end the experimental period 20 chickens from each group have been sacrificed then weighed in order to determine the carcass yield. Two types of yields have been calculated: weight of fat/weight of the carcass and weight of carcass eviscerated/weight of carcass non-eviscerated. The carcass yield permits to measure the probiotic effect on the quality of the carcass.

The number of white blood cells, the serum cholesterol and triglycerides concentration have been determined by blood withdrawals done on 80 chickens chosen randomly from each treatment group.

The statistical analysis has been performed using ANOVA.

RESULTS AND DISCUSSION

1.1. Animal production performance

Results of production performances are summarised in Table 2. The evolution of the live weight of the Experimental group is marked, from the sixth week, by a significantly higher live weight than the Control (1703.67±34.4 vs. 1574.11±33.39 g). The average live weight at the end of the

experimental period is 2586.48 g and 2252.79 g for the (Exp.) and (Cont.) group respectively, which corresponds to an improvement of 12.89%.

These results agree with the works of Cavazonni *et al.*, (1998) and Stella (2005). Kabir *et al*., (2004) observed an improvement of the chickens' weights with other probiotics, however Karaoglu and Dardug (2005) did not establish any effect with *Saccharomyces cerevisiae*.

During all raising phases, chickens having received a supplemented diet with *P. acidilactici* presented feed ratios lower than the Control (Table 3). At the eighth week, chickens of the (Cont.) group had a feed ratio slightly higher than that of the (Exp.) group (2.45 vs. 2.37) respectively. Studies done by Pelicano *et al.,* (2004); Silva *et al.*, (2000); Franco *et al.*, (2005) demonstrated an improvement of the feed ratio with chickens fed on probiotics such as *Bacillus subtilis*, *Lactobacillus acidophilus*, *Saccharomyces cerevisiae* and *Enterococcus faecium*. Johri (2004) did not observe any positive effect on the feed ratio of the chickens when *Streptococcus lactis* was incorporated in the feed.

The mortality rate in the two treatment groups is almost identical (6.57 vs. 6.51). Siwicki *et al.*, (2005), Ramirez (2005) proved a reduction of the mortality rate due to the addition of probiotics in feeds of chickens.

Results concerning the carcass yield and the abdominal fat are summarised in Table 4. There was a clear influence of the use of *P. acididilactici* on the final quality of chickens' carcasses, a significant improvement ($p \leq 0.01$) of the carcass yield is noted (60.40 vs. 66.32%) for (Cont.) and (Exp.) respectively. However there was no significant reduction in the abdominal fat yield for the (Exp.) group in relation to the (Cont.) (1.90 vs. 2.27%). Kalavathy *et al.*, (2003, 2006); Miazzo *et al.*, (2005) observed a significant reduction of the abdominal fat content of the chickens, whereas Pelicano *et al.*, (2004) and Arslan (2004) did not observe any effect of probiotics on the carcass yield of the chickens.

1.2. White blood-cells count

The number of white blood cells has been influenced by the addition of the probiotic in the diet. A significant difference ($p \leq 0.01$) has been observed between the (Cont.) group (25260 ± 3258 /mm^3) and the (Exp.) group (30365 ± 3210 /mm^3). (Table 3)

1.3. Serum lipids concentration

The analysis of serum lipids' concentration of the broiler chickens is summarised in the table 5. The content in lipids of blood that is represented by triglycerides and cholesterol is reduced in a significant manner ($p \leq 0.01$) in the group of chickens receiving *P. acidilactici*, during all raising phases. This could be explained by the fact that probiotics may possess the property of reducing cholesterol in the blood, which is due to the inhibition of the hepatic synthesis of cholesterol, and to their capacity of déconjuguating the biliary salts (Mercenier *et al.*, 2002; Pereira *et al.*, 2003; Lim *et al.*, 2004). On the other hand, Kanashiro *et al.,* (2001) and Djouvinov *et al.*, (2005) did not observe any variations of cholesterol and triglycerides content in chickens' blood while using mixture of different strains of probiotics (*lactobacillus sp, bacillus sp, enterococcus faecium, streptococcus thermophilus*) in the diet.

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Table 1. Composition of the broiler chicken feeds (%)

Ingredients	Starting phase (d0–d21)	Growing phase (d22–d42)	Finishing phase (d43–d56)
Maize	58	60	60
Soyameal	30	25	18
Cereals by-products	9	13	18
CMV*	1.5	1	1
Bicalcic phosphate	1.5	1.5	1.5
Chemical chimique			
ME kcal /kg	3040	3100	3180
Crude protein	21.500	18.500	17.500
Fiber	3.066	2.770	2.536
Ash	7.50	6.20	6.00

*CMV : mineral vitaminic complement

Table 2. Evolution of the live weight (g) of broiler chickens in control and experimental groups

Age (days)	Control group (n= 200)	Experimental group (n =200)	P
0	46.11±0.20	44.08± 0.25	NS
14	241.88± 3.33	245.45± 3.61	NS
28	802.36± 15.06	842.97± 21.44	NS
42	1574.11± 33.39	1703.67± 34.4	*
56	2252.79± 24.50	2586.43± 27.6	*

NS : not significant
*($p \leq 0.01$)

Table 3. Feed ratio, mortality rate, number of white blood cells of the broiler chickens in control and experimental groups at day 56

	Control group	Experimental group	P
Feed ratio	2.45	2.37	NS
Mortality rate %	6.57	6.51	NS
Number of white blood cells (n/mm^3)	25260±3258	30365±3210	*

Table 4. Carcass yield of broiler chickens in the control and experimental groups

	Control group (n=20)	Experimental group (n=20)	P
Live weight (g)	2285.57± 48.00	2629.90±45.20	*
Carcass weight (g)	1715.56±38.80	2091.84± 44.90	*
Carcass yield (%)	60.40	66.32	*
Fat weight (g)	37.36±5.66	39.92±4.42	NS
Fat Yield (%)	2.27	1.9	NS

NS : Not significant
* : ($p \leq 0.01$)

Table 5. Serum lipids' concentration in the of broiler chickens in the control and experimental groups

Parameters		Ages (n=80)				P
		d14	d28	d42	d56	
Cholesterol (g/l)	Exp.	1.10± 0.06	0.94± 0.09	0.93± 0.05	0.84± 0.09	*
	Cont.	1.20± 0.01	1.13± 0.01	0.96± 0.12	1.09± 0.11	
Triglycerides (g/l)	Exp.	1.42 ±0.07	1.23± 0.04	0.86± 0.08	0.84 ±0.06	*
	Cont.	1.46± 0.09	1.25± 0.10	1.15 ±0.03	0.86 ±0.06	

NS : Not significant
*: ($p \leq 0.01$)

THE NUTRITIVE VALUE OF YEAST *SACCHAROMYCES CEREVISIAE* ENRICHED IN COPPER, IRON AND MANGANESE

Dobrzański, Z.[1], Opaliński, S.[1], Dolińska, B.[2], Chojnacka, K.[3] and Kołacz, R.[1]

[1] Wroclaw University of Environmental and Life Sciences, Poland;
[2] Silesian Medicine University of Katowice, Poland; [3] Wroclaw University of Technology, Poland

SUMMARY

Dietary yeast enriched in bioelements like Cu, Fe and Mn was produced on the basis of *Saccharomyces cerevisiae* and whey. Optimal conditions of incubation, temperature and the concentrations of whey, salts (cupric acetate, manganic sulfate and ferric sulfate) were determined. The content of proteins, fat, fibre, ash and the concentrations of macroelements and 47 trace elements were determined in dry matter of copper, iron and manganese yeast. Moreover, the content of bacteria and fungi in yeast were examined after 3 months of their storage. There were very effective bioavailability of Cu and Mn both for young fatteners and laying hens.

Keywords: *Saccharomyces cerevisiae*, dietary yeast, nutritive value, copper, iron, manganese

INTRODUCTION

From the beginning of the last century, yeast has been used in animal feeding as a rich source of well digested protein as well as vitamin B and some important bioelements. The significance of yeast, as a feed additive for farm animals, rapidly increase when the use of animal meals and also antibiotics were strictly forbidden. Active dry yeasts (95% of dry matter), living yeast culture in the form of prebiotics or products of yeast origin are often used [2,7]. The bakery yeast, *Saccharomyces cerevisiae*, are produced on industrial scale from molasses but also from whey and from waste of starch, fat and other organic raw materials. Many scientific researches proves, that it is possible to enrich yeast in macroelements or even trace elements, that could be useful in reduction or even in elimination of mineral premixes (inorganic form of elements) from animal feeding [3,10,11,14].

The waste-free technology production of dietary yeast enriched in selenium, zinc and chromium was elaborated in Poland in last years. In defined incubation conditions it is possible to enrich yeast in many bio elements [4,12].

The aim of this work was to estimate the chemical composition of yeast *Saccharomyces cerevisiae* enriched in Cu, Fe and Mn as well as their nutritive value, especially taking into consideration the content of macro and micro elements.

MATERIALS AND METHODS

Detailed description of the investigated yeast production method was presented in previous publication [5] and patented in Patent Office RP.

The samples of the yeast Saccharomyces cerevisiae enriched in copper (Y-Cu), iron (Y-Fe) and manganese (Y-Mn) were analyzed to determine:

- the content of water, crude protein, crude fat, crude ash, crude fibre – with the use of the generally accepted procedures [1],
- the content of amino acids – with the use of the automatic amino acids analyser AAA-400 (Ingos – Czech Republic),
- the content of the micro and macroelements – with the use of mass spectrometry with inductively coupled plasma (ICP-MS) by instrument Varian UltraMass-700 (prod. Australia)[8],

the content of bacteria and fungi in the fresh yeast and after 3 months of storage in room temperature – according to polish norms (PN-R-64791 and PN-ISO-7954).

Statistical assessment of the results was performed with the use of "Statistica for Windows 5.1" (StatSoft Inc. 1997).

RESULTS AND DISCUSSION

The composition of the examined yeast Y-Cu, Y-Fe and Y-Mn generally meet the requirements defined in PN-81/A-79006 or described by Smulikowska and Rutkowski [13], only the content of crude protein and crude ash were lower than in standards (tab.1). Whereas the amino acids composition was different from the standards but it was easy to explain when we consider the differences in yeast's technology production. The highest concentrations of amino acids were determined for aspartic acid, glutamic acid, lysine and the lowest for cysteine and methionine (tab. 2). Taking into consideration the physiology of digestion, the highest lysine content was a very good result, because this essential amino acid is limiting the value and quality of protein in animal feeding [9].

The microbiological tests of examined yeast showed very low contamination, the content of micro organisms were below the limits defined in the standards (PN-R-64791). The content of bacteria (aerobic mesophiles) were between 2×10^2 and $3{,}4 \times 10^3$ cfu/g in the fresh material and between 3×10^3 and $4{,}4 \times 10^5$ cfu/g in the material after storage in the room temperature (3 months). The fungi were not detected both in the fresh material and in the yeast after storage.

The table 3 presents that the dominant macroelements were phosphorus and magnesium and the lowest concentration was found for sodium. Total concentration of macroelements was the highest for Y-Fe and the lowest for Y-Cu.

The content of the most important microelements in the examined yeast show tables 1 and 4. The highest concentrations were determined for Fe, Mn and Cu (maximal ca. 2%), that proves that the use of the enriched yeast for the premix production or for the production of biopreparations (supplements of diet) could be very good opportunity. The highest concentrations, except for Fe, Mn and Cu, were detected for zinc (ca. 200 mg/kg), silicon, boron, aluminium and rubidium. The concentrations of Cr, Se, Co, Cs, Ga, Ge, Mo, Sb, Ti, V and Zr were detected under the level of 1 mg/kg, the lowest concentrations were determined for nickel, bismuth, tin and lithium (under the detection threshold).

It was found that from the trace elements the highest concentrations were determined for uranium (Y-Cu), indium (Y-Fe), thorium (Y-Fe) and lanthanum (Y-Cu) and the concentrations of Be, Hf, Nb, Os, Pt and Re were under the level of 5 μg/kg. It was interesting that the composition of trace elements in each group of yeast (Y-Fe, Y-Cu, and Y-Mn) were different from each other despite the fact that the substrate for the yeast production (whey) was the same.

It is also worth to say that the concentrations of toxic metals (As, Cd, Hg, F, and Pb) in the examined yeast were under acceptable levels. The highest concentrations of lead and cadmium were determined, respectively, in Y-Fe and Y-Mn.

The obtained results concerning the composition of the yeast were very difficult to compare with the results of other authors because the technology of production and the substrates were different. However, the results of Fernandes et al. [6] showed that the fodder yeast, obtained in the process of the ethanol production from the sugar cane, had very high concentrations of macro and micro elements especially of sodium, potassium and zinc. The investigation of the Biocer® yeast (enriched in Se, Zn, Cr) composition showed that, except of the enriching elements, the highest concentrations were determined for P, Na, Ca, Mg and Fe. Besides, the presence of 30 micro and trace elements were detected, the concentrations of some of the elements were similar to the concentrations of elements which were determined for Y-Cu, Y-Mn and Y-Fe [4].

The nutritional examination made on young fatteners and laying hens showed very good bioavailability of Cu and Mn, worse of Fe, from Y-Cu, Y-Mn and Y-Fe. The results of the bioavailability researches will be published in the separate publication in the next future.

CONCLUSIONS

The yeast *Saccharomyces cerevisiae* enriched in Fe, Mn and Cu were characterized by very good chemical properties, high content of protein, beneficial composition of amino acids and elements. Therefore, the investigated yeast could be very useful in feeding (feed additives) for monogastric animals

ACKNOWLEDGEMENTS

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Table 1. The basic composition of the yeast *Saccharomyces* cerevisiae enriched with Fe, Mn and Cu

Item	**Y-Fe**	**Y-Mn**	**Y-Cu**
Dry matter [%]	96,2	95,3	95,2
Crude ash [%]	6,62	6,81	5,09
Crude protein [%]	38,0	39,75	39,32
Crude fat [%]	1,01	0,93	0,83
Crude fibre [%]	trace	trace	trace
Metabolic Energy [MJ/kg]	10.33	10.48	10.16
Bioelements (g/kg)			
Fe	1,44–26,45*	0,07	0,026
Mn	0,012	5,33–19,96*	0,011
Cu	0,004	0,004	0,43–18,49*

*maximal values

Table 2. The amino acid composition (g/kg) of the yeast *Saccharomyces cerevisiae* enriched with Fe, Mn and Cu

Amino acid	Y-Fe	Y-Mn	Y-Cu
Asp	40,05	39,56	37,54
Thr	19,27	18,95	19,00
Ser	18,48	17,89	19,18
Glu	46,18	47,02	45,47
Pro	15,76	17,23	15,18
Gly	17,61	18,61	17,70
Ala	22,68	24,27	23,55
Val	22,06	23,15	21,86
Ile	18,59	19,61	18,13
Leu	27,12	29,14	27,80
Tyr	13,11	13,05	10,69
Phe	16,17	16,89	16,19
His	12,06	11,54	10,99
Lys	31,84	31,31	34,48
Arg	21,63	23,51	21,74
Cys	4,23	3,98	4,40
Met	4,57	4,75	4,73

Table 3. The content of macroelements (%) of the yeast *Saccharomyces cerevisiae* enriched with Fe, Mn and Cu

element	Y-Fe	Y-Mn	Y-Cu
Ca	0,210	0,245	0,130
P	1,052	0,990	0,653
Mg	0,873	1,042	0,329
K	0,757	0,659	0,641
Na	0,044	0,003	0,002
S	0,402	0,218	0,196
Total	3.338	3,157	1,951

Table 4. The content of microelements (mg/kg) of the yeast *Saccharomyces cerevisiae* enriched with Fe, Mn and Cu

element	**Y-Fe**	**Y-Mn**	**Y-Cu**
Cr	0,86	6,0	0,29
Zn	221	193	213
I	1,91	0,67	0,082
Se	0,14	0,20	<0,10
Ag	0,069	0,65	0,23
Al	7,6	10,7	8,03
B	25,0	24,6	22,6
Ba	0,88	1,28	0,582
Bi	<0,0005	<0,0005	0,027
Co	0,12	0,205	0,162
Cs	0,030	0,038	0,035
Ga	0,12	0,23	0,17
Ge	0,78	0,08	0,12
Li	<0,27	<0,27	<0,27
Mo	0,095	0,092	0,035
Ni	<0,01	1,20	<0,01
Sb	0,025	0,013	0,020
Sc	1,52	1,50	2,44
Si	52,4	52.0	47,5
Sn	<0,004	<0,004	1,46
Sr	5,6	6,4	3,0
Rb	8,8	9,3	8,5
Ti	0,71	0,93	0,55
V	0,290	0,311	0,168
Zr	0,053	0,075	0,071

FEATURES OF REGULATOR PROCESSES OF QUANTITY OF SYNANTHROPIC RODENTS DURING IMPLEMENTATION OF DERATIZATIVE WORKS

Dvylyuk, I. and Demchuk, M.

Lviv National Academy of Veterinary Medicine named after S.Z. Gzhytskyi, Pekarska, 50, 79010, Lviv, Ukraine, dvylyuk@rambler.ru

ABSTRACT

In the article are given the dynamics of control processes in number of rodents during usage of different deratizational methodical approaches. The analysis of autoregulational processes of rodent population under conditions of artificial regulation of their number is made and the expediency of complex deratizational by means of modern technologies is given.

Keywords: deratization, acoustic repellents, population autoregulation

INTRODUCTION

Necessity of artificial decrease in number of some dangerous rodents by means of traditional forms and means of fight doesn't allow achieving desirable effect. Widely used practice of one moment influence on population of synanthropic rodents causes death of rodents but it doesn't provide a lasting effect. During a short period of time reinfestation of the object by rodents takes place. Nowadays it is very difficult to define the minimal influence on biotope and rodents inhabiting it die. No correlations or general peculiarities were revealed. In 1972 Davis D.E. taking into account reasons of renewing of synanthropic rodents after usage of poison substantiated necessity of alternative method in fight with rodents. He considered the realization of complex program of deratization works to be the most important for some methods help to present occurrence of rodents. A good example of such approach is complex usage of existing means of light with rodents, acoustic repellent and chemical preparations in particular. Allow mentioned concept gives possibility to decrease costs during deratization and increase its effectiveness. It is well known that homeostasis of population structures plays an important role in maintaining of optimal of population. It would be wise to observe such processes according to the (Figure 1).

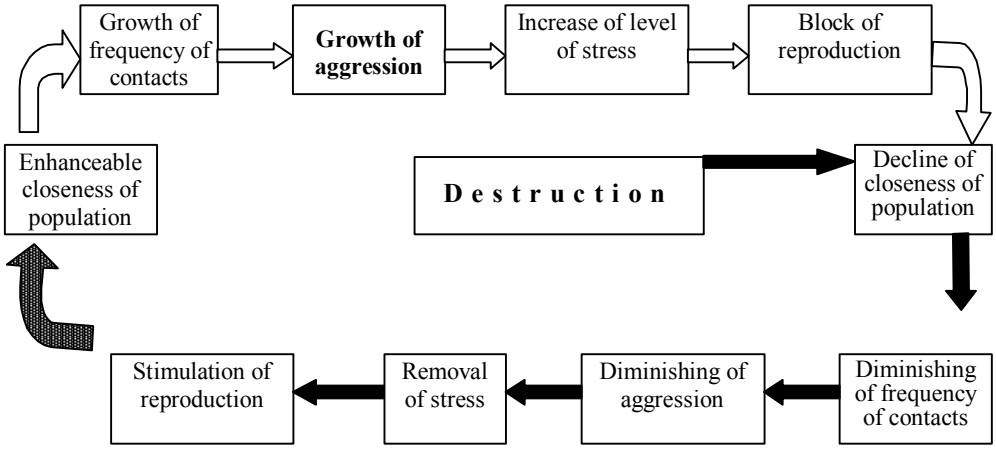


Figure 1. Scheme of population autoregulation of quantity of rodents

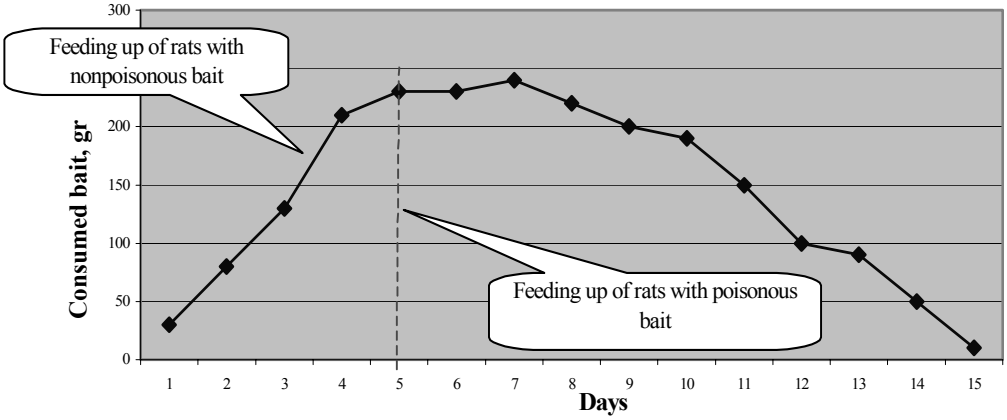
Necessity of solving the problem of quantity of rodents was the main thing which made us make investigations.

MATERIAL AND METHODS

Establishment of infestation level and monitoring of dynamics intensity of occupying by rodents the objects was performed according to the methods of Kolbushevskyy. Deratization measures were performed at the farm ‘’Lvivske’’ using poisoned decoy and acoustic repellent AP-010. At first rodents were given nonpoisonous bait to reveal the number of rodents. As bait are used feed given to swine (combined feed) to develop such phenomenon as aversion to food. Bait was placed for 5 days at the same place in the bait-station it was weighed every day. For deratization we used preparation from the group of anticoagulants – racumin with the prescription for use. Bait was placed in the form of mixtures: 5% of racumin with feed (the weight was 300 gr.). Bait was prepared according to the following recipe (racumin 50 g, combined feed-930 g, oil 20 g). Deratizational works were performed for 15 days and the results were studied 15 days later.

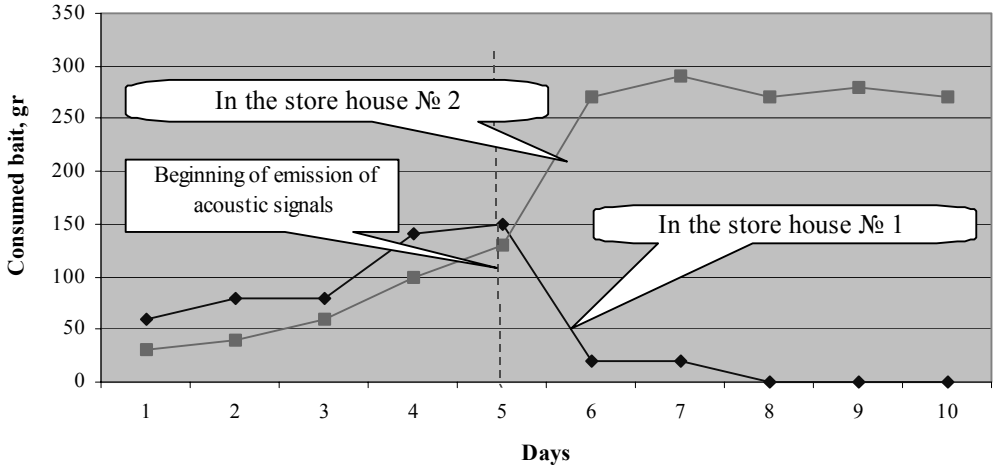
RESULTS OF THE INVESTIGATION

Investigations were carried out at the sty of the enterprise ‘’Lvivskie’’ of the total area 756 square meters. According to calculations and taking into account consumed bait and area of the sty the number of rodents was 24 and intensity of occupation-3,17 (24x100/756) and it may be classified as medium intensity(Graphic 1).



Graphic 1. The dynamics in bait consumption of the enterprise ''Lvivskie''

Picture shows that feeding up of rats with nonpoisonous bait for 5 days allowed to increase its consumption by 7,7 times, to reveal the occupation of the objects by rodents and also to place deratizational bait stations such places were bait was the most popular. Consumption of poisonous bait began from a large number and stopped 10 days later. Although visiting of deratizational bait station by rats stopped serving personnel noticed movement of rodents in the farm which was very dangerous from the economical, epizootic and epidemiological point of view. According to calculations taking into consideration total number of consumed bait and area quantity of rodents was 15(150/10) and intensity of their occupation-4,2(15x100/900) which can be classified as medium intensity of occupation. Usage of different bait favoured the increase of their consumption activity both in store house № 1 and in store house № 2 (Graphic 2) and was much higher at the 5-th day compared with the first day by 2,5 and 4,3 times respectively.



Graphic 2. The dynamics in bait consumption of the enterprise ''Lvivskie'' in store house no 1 and 2

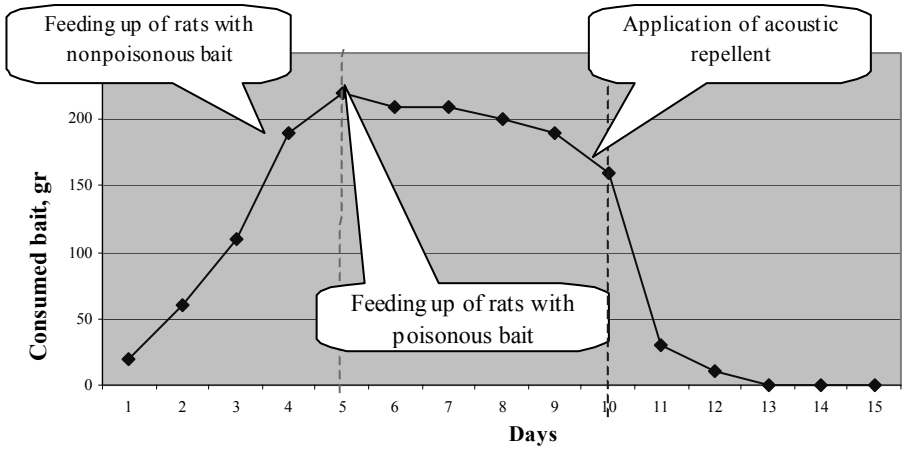
Usage of acoustic repellent AP-010 in store house № 1 led to dramatic decrease in consumption of bait to 13,3% during the first day of emission and its full stopping of consumption during the third day. According to the calculations, taking into account total quantity of consumed bait and area of store house № 2 in " Lvivskie" enterprise revealed number of rodents before action of repellent was 13 (130/10) and intensity of their occupation was 3,6(19x100/360), with may be classified as medium intensity of occupation. After action of the device AP-010 in the store house there was noticed an increase in number of rodents and also intensity of their occupation up to 29 (290/10) and 8,19x100/360).

Analyzing the dynamics in bait consumption in both store houses № 1 and № 2 we observed the escape of rodents in store house № 1 and rapid increase of their number in store house № 2 after action functioning of acoustic repellent.

As a confirmation we observed rapid increase during one day, of consumed bait in the store house № 2 by 2,1 times and increase of occupation intensity by rodents by 2,3 times.

Obtained results showed that usage of acoustic repellent AP-010 made rats leave the place where the acoustic repellent was installed. But repellent action causes the migration of rats to those places where they can safely exist. It is not wise to use acoustic repellents as the only means with the aim of destruction and release from rodents. In such cases it would be wise to use chemical means as a destructive measure along with installation of acoustic repellent.

According to calculations and taking into account total quantity of consumed bait and area of the sties revealed number of rodents was 22(220/10) and intensity of their occupation was 6,4 (22x100/342) which may be classified as large intensity of occupation (Graphic 3). Individuals of rodents' populations were extremely active in consumption of bait on the fifth day. On the 10-th day consumption of bait with poison decreased on 27,3% and that may be the beginning of the process of poisoning. Usage of acoustic repellent during the first day of emission decreased the bait consumption on 81,2% and during the third day we didn't observe any activity in bait consumption.



Graphic 3. The dynamics in bait consumption of the enterprise PAF''Franko''

Effectiveness of deratizational works was defined by means of calculations of consumed bait, quantity of opened holes and from the information of the farmers 15 days later, after destructive works.

During 5 days there was resealed an increase in ground activity according to the information of operating staff and rats managed to open there holes which were closed with the help of the soil. We also revealed consumption of bait in small numbers which were as much as 60 gr. Obtained results may confirm the appearance of new generation of individuals or migrating rats. But such low level of consumption of nonpoisonous bait doesn't mean that the object got rid of rats from former generation. In store house № 1 we didn't observe any consumption of bait and activity of rats was not revealed but increase of their land activity and consumption of bait in store house № 2 were noticed.

From the moment of completion of destructive works and installation of acoustic repellents AP-010 during the period of deratizational works effectiveness we didn't reveal any land activity or consumption of bait by rats.

It should be mentioned that usage of methods for definition of quality of deratization as to the number of consumed bait was not acceptable by us for development of taste aversion caused refusal from bait of rodents in the population and that was clearly observed in the farm "Lvivske".

Summarizing obtained results we may suppose that separate usage of poisonous bait and acoustic repellent AP-010 doesn't allow to obtain steady and desirable effect but only their combined usage according to the complex plan allows raising the effectiveness of fight with dangerous rodents at the objects of veterinary and sanitary control.

Explanation of such effectiveness may be the combination in the complex plan deratization of wide range to specific mechanisms in biological systems both at the individual and population levels, directed at preservation of rodents.

It is displayed in the formation of aversion and discomfort situation for rodents at the objects with the help of the poison as destructive means and repellent not only as aggressive measure but also informative means about danger. Taking into account high protective and adjustive peculiarities of rodents the latter is of so great important when its usage is connected with death of individuals and may lead to fixing of the characteristics of this signal even at the reflector level. In such cases usage of poison minimizes the number of rodents and repellents guarantee constant maintenance of obtained effect, at the object and warning of their appearance.

CONCLUSIONS

Nowadays chemical means can reduce a number of rodents only for a short period of time but they don't affect the integrity of their population structures, but in future it would be wise to use such approaches under which complete structural and functions of populations could ensure lasting decrease in number of rodents to minimum. Usage of chemical means along with acoustic repellents increases effectiveness of deratizational works, ensuring getting rid of rodents for a long period of time. It is economically profitable and scientifically grounded to apply chemical and acoustic means of deratization on the basis of complex purpose – oriented plan.

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ACUTE PHASE PROTEIN RESPONSE IN PIGS EXPERIMENTALLY INFECTED WITH *HAEMOPHILUS PARASUIS*

Fablet, C., Marois, C., Eono, F., Kobisch, M. and Madec, F.

French Food Safety Agency, Zoopôle Les Croix, B.P. 53, 22 440 Ploufragan, FRANCE

SUMMARY

The aim of the trial was to look at the course of the serum concentration of the acute phase proteins C-Reactive Protein, Haptoglobine and Serum Amyloïde A after infection of SPF pigs with a strain of *Haemophilus parasuis*. The results indicated that APP response followed up clinical symptoms of the outcome of the disease. Hp, CRP and SAA seems to be sensitive and non specific bio marker of clinically and subclinically infected pigs with Glässer's disease. The determination of these protein concentrations may be a useful tool to distinguish acute from chronic phases.

Keywords: pigs, *Haemophilus parasuis*, acute phase response, C-Reactive Protein, haptoglobine, serum Amyloïd A, experimental trial

INTRODUCTION

The acute phase response is an unspecific systemic reaction of the organism that occurs shortly after infection, inflammation or trauma and includes changes in the concentration of plasma proteins called acute phase proteins (APPs) such as Haptoglobin (Hp), C-Reactive Protein (CRP) and Serum Amyloid A (SAA). Quantification of their concentrations in plasma or serum of pigs could provide valuable diagnostic information for prognosis and monitoring disease (Eckersall, 2000). In recent years, a growing interest in infections caused by Hps in pigs has been identified due to the severity of the disease when the infectious agent is introduced in high health status herds (Rapp-Gabrielson et al., 2006). Even if the evolution of the acute phase response of pigs induced by experimental infections has been documented for some bacteria or virus, to the best of our knowledge, no trial related to the APPs behaviour following Hps infection has been reported. The aim of the trial was to look at the course of the serum concentration of the acute phase proteins C-Reactive Protein (CRP), Haptoglobine (Hp) and Serum Amyloïd A (SAA) after infection of pigs with a strain of *Haemophilus parasuis* (Hps).

MATERIALS AND METHODS

A total of 16 specific pathogen free pigs (SPF) was used for the assay. They were divided into 4 groups that differed in the age and route of infection:

- **Group 1**: two 10-week-old pigs were not inoculated and constituted the control group,
- **Group 2**: six 10-week-old pigs intranasally infected with $2x10^8$ colony-forming units (CFU) of Hps,

–**Group 3**: four 7-week-old pigs intranasally infected with $2x10^8$ CFU of Hps,
–**Group 4**: four 7-week-old pigs infected by intratracheal injection with $2x10^8$ CFU of Hps.

On day 0, the pigs were inoculated with a strain of Hps isolated from septicaemia. The pigs were monitored daily before and after challenge by measuring rectal temperature and recording specific clinical symptoms such as lameness, swollen joints, dyspnoea and nervous signs (tremor). Blood samples were obtained at days 0, 7 and 14 for determination of CRP, Hp and SAA concentrations. On day 14 after challenge, survival pigs were euthanized and necropsied. Macroscopic lesions were recorded. At necropsy, nasal swabs, tonsillar, lung and arthritic joints samples of every pig were taken and submitted to PCR analysis for Hps detection (Olivieira et al., 2001). Serum levels of CRP, Hp and SAA were measured with commercial assay kits (Tridelta Development, Greystones, Ireland).

RESULTS

Fever was observed in all infected groups within day 01 and 09 p.i. In group 2, 2 pigs showed a rectal temperature above 40.5°C. One pig had elevated temperature on days 01 and 02 p.i. and died soon after. The second pig developed fever on day 04 p.i. and was euthanized on day 05 p.i. In group 3, 2 pigs had elevated body temperature on days 01–05 p.i. and were euthanized on days 02 and 05. Fever concerned 2 pigs in group 4. One pig died on day 02. The other pig had a body temperature exceeding 40°C during 7 days after the challenge (days 03–09). Mortality was observed during the first week following the challenge (day 02 to day 07). Lameness was identified after infection whatever the age and route of infection. The number of affected pigs was highest for the group intranasally infected at 7 weeks-old (3/4 pigs, group 4). In this group, nervous signs were recorded in one pig (Table 1).

Table 1. Clinical signs observed in SPF pigs after experimental infection with a strain of *Haemophilus parasuis* (14 pigs, group 2: 10-weeks-old pigs intranasally infected with $2x10^8$ colony-forming units; group 3: 7-weeks-old pigs intranasally infected with $2x10^8$ colony-forming units; group 4: 7-weeks-old pigs infected by intratracheal injection with $2x10^8$ CFU)

Clinical observation	**Group 2**		**Group 3**		**Group 4**	
	No of affected pigs/No of inoculated pigs	Day p.i.	No of affected pigs/No of inoculated pigs	Day p.i.	No of affected pigs/No of inoculated pigs	Day p.i.
Lameness	1/6	3–4	1/4	3–5	3/4	2–14
Nervous signs	–	–	–	–	1/4	2

At necropsy, polyserositis was observed in all infected groups (Table 2). Macroscopic lesions were more frequent in pigs intranasally infected at 7 weeks of age. In this group, only one pig was free from gross lesions whereas 2/4 pigs and 5/6 pigs had no visible lesions in group 3 and 4 respectively. Hps was detected from nasal and tonsillar samples of all infected pigs, indicating that all pigs have been contaminated.

Table 2. Pathological findings at necropsy in 3 groups of SPF pigs infected with a strain of *Haemophilus parasuis* (14 pigs, group 2: 10-weeks-old pigs intranasally infected with $2x10^8$ colony-forming units; group 3: 7-weeks-old pigs intranasally infected with $2x10^8$ colony-forming units; group 4: 7-weeks-old pigs infected by intratracheal injection with $2x10^8$ CFU)

Lesion	Nbre of affected pigs/Nbre of inoculated pigs		
	Group 2	**Group 3**	**Group 4**
Pleuritis	1/6	2/4	–
Peritonitis	1/6	2/4	2/4
Arthritis	1/6	3/4	2/4
Pneumonia	–	1/4	1/4
Pericarditis	–	2/4	–
Total	**1/6**	**3/4**	**2/4**

Individual CRP, Hp and SAA responses of the pigs are given Figures 1, 2, 3 and 4 for groups 1 to 4 respectively. Since 5 pigs died before day 07 p.i., their acute phase responses were not investigated. Dealing with the remaining pigs, one pig in group 4 (247) developed an acute phase response 7 days p.i. CRP level showed a 25-fold increase as compared to day 0. Hp and SAA concentrations rose respectively from 0.03 to 7.15 mg/ml and 24.41 to 1094.86 µg/ml. High levels of CRP and Hp were still observed 14 days p.i. SAA decreased more rapidly to reach 80.2 µg/ml, 14 days after challenge. To a lesser extent, a slight increase of Hp level was observed in one pig (248) in group 3 between days 7 and 14 p.i. (0.07 mg/ml to 1.39 mg/ml). When correlating responding APPs with clinical signs and pathological findings, the pig 247 showed fever from days 04 to 06 p.i. and lameness until the end of the trial (day 14 p.i.). At necropsy, polyserosits was observed (peritonitis and arthritis). Pig 248 suffered from arthritis. All infected pigs which did not showed severe fever or clinical symptoms between days 0 to 14, or macroscopic lesions at necropsy, had acute phase proteins levels quite similar to those of control pigs.

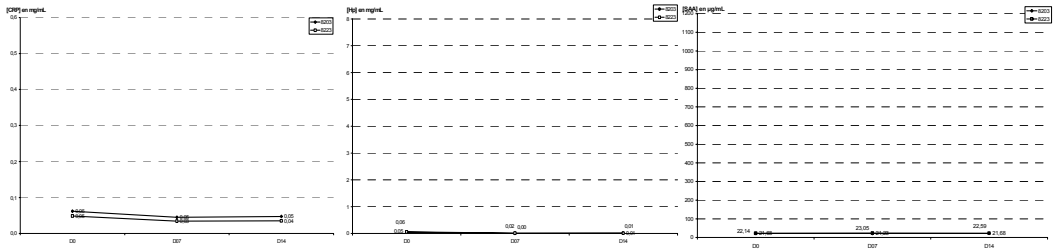

Figure 1. Concentrations of C-Reactive Protein (A), Haptoglobine (B), Serum Amyloid A (C) in serum of individual control pigs before and on various time-point after inoculation with *Haemophilus parasuis*

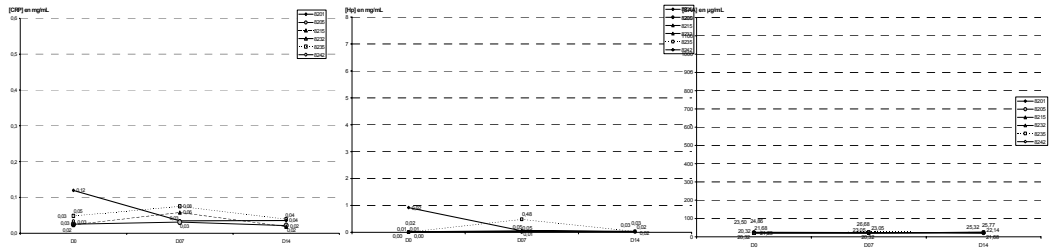

Figure 2. Concentrations of C-Reactive Protein (A), Haptoglobine (B), Serum Amyloid A (C) in serum of individual pigs of group 2 before and on various time-point after inoculation with *Haemophilus parasuis*

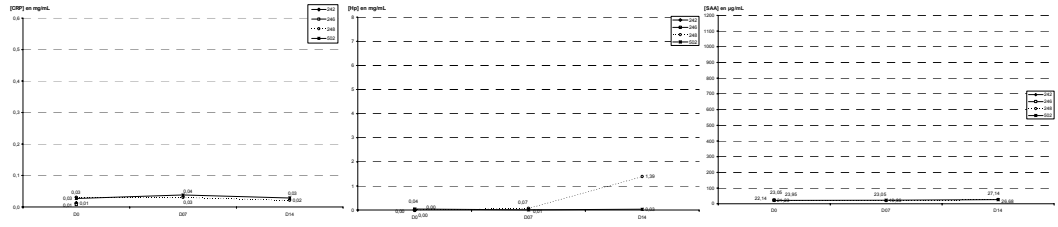

Figure 3. Concentrations of C-Reactive Protein (A), Haptoglobine (B), Serum Amyloid A (C) in serum of individual pigs of group 3 before and on various time-point after inoculation with *Haemophilus parasuis*

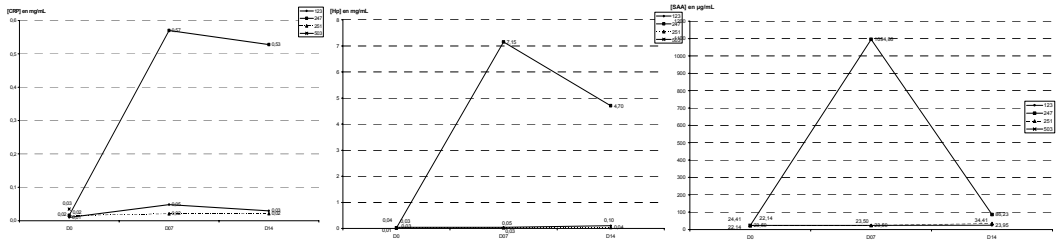

Figure 4. Concentrations of C-Reactive Protein (A), Haptoglobine (B), Serum Amyloid A (C) in serum of individual pigs of group 4 before and on various time-point after inoculation with *Haemophilus parasuis*

DISCUSSION-CONCLUSION

The *Haemophilus parasuis* strain used for this trial was isolated from a pig affected by Glässer's disease. Experimental infection of SPF pigs with this strain by nasal or intratracheal route induced for 7 out of 14 infected pigs, clinical (fever, lameness, nervous signs) and typical macroscopic lesions (polyserositis) of the disease. Furthermore, mortality occurred within the first days after challenge as described in the acute phase of the disease (Oliveira and Pijoan, 2004). Half of the

infected pigs did not developed clinical signs, especially an severe increase of body temperature. Nevertheless, Hps was detected in the upper respiratory tract of this pigs, indicating a colonisation of the mucosa. An acute phase response was observed 07 and 14 days post infection in pigs showing clinical signs of Glässer's disease during the trial. On the other side, no significant changes in the CRP, Hp and SAA concentrations were noticed at these time for pigs carrying *Haemophilus parasuis* in the upper respiratory tract and free from clinical signs and macroscopic lesions. These results indicated that APP response clearly followed the clinical symptoms of the disease. Hp, CRP and SAA seem to be sensitive and react as non specific bio markers of clinically and subclinically infected pigs. The determination of the concentrations in these proteins may be useful to distinguish acute from chronic phases.

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THE EFFECT OF MICRO-CAPSULATED YEAST SUPPLEMENTATION ON RUMEN FERMENTATION IN SHEEP*

Jurkovich, V.[1#], Kutasi, J.[2], Fébel, H.[3], Brydl, E.[1], Könyves, L.[1] and Tirián, A.[1]

[1] Szent István University, Faculty of Veterinary Science, Department of Animal Hygiene and Herd Health, Budapest, Hungary; [2] Dr. Bata R&D Co., Ócsa, Hungary,
[3] Research Institute of Animal Breeding and Nutrition, Herceghalom, Hungary;
[#] E-mail: jurkovich.viktor@aotk.szie.hu

SUMMARY

The aim of our study was to investigate the effects of trehalose producing yeast supplementation on the rumen fermentation of sheep. The experimental design was 3x3 Latin square, using 9, rumen cannulated merino wethers. Group A received trehalose producing yeast (Live-Sacc Dairy), group B received trehalose non-producing yeast supplementation (Live-Sacc), mixed in the ration, group C, without supplementation, served as control. Samples of rumen fluid were taken before and after feeding. Rumen pH, ammonia and VFA concentrations were measured. Ammonia concentrations remained unchanged in the control group, significantly decreased in both groups receiving supplementation. TVFA-concentration increased after feeding in all groups.

Keywords: *Saccharomyces cerevisiae*, sheep, rumen fermentation

INTRODUCTION

Saccharomyces cerevisiae (Sc) yeast strains are widely used as additives in the feeding of ruminants, dairy cows primarily. Being a possible alternative for ionophore antibiotics in growth promotion and other favourable effects have put Sc supplementation in the focus of research. Effects of Sc in vivo and in vitro may differ. Several studies have lead to contradicting conclusions. Such variety of experimental results is presumably due to differences in dosage of the additive, composition of rations or viability of the strains used.

Our previous studies have shown that the intraruminal viability of Live-Sacc Dairy, meaning micro-capsulated *Saccharomyces cerevisiae* NCAIM 1286, improved as a result of increased trehalose producing activity.

The aim of our study was to investigate the effects of micro-capsulated trehalose producing yeast (Live-Sacc Dairy, LSDairy) supplementation on the rumen fermentation of sheep.

* Research was supported by National Office for Research and Development (OMFB-1213/2004)

MATERIAL AND METHODS

Animals and diets

The experimental design was 3x3 Latin square, using 9, rumen cannulated yearling merino wethers (*Table 1*.). The sheep were kept individually in cages within sight and auditory communication. The animals were fed twice a day (at 8 am and at 4 pm) with a ration of 800 g meadow hay and 500 g lamb concentrate. Licking salt in blocks and water were provided ad libitum. Group A received trehalose producing yeast (Live-Sacc Dairy) in the amount of 2,5 g mixed in the daily feed. Group B received trehalose non-producing yeast supplementation (Live-Sacc) in the amount of 2,5 g mixed in the ration. Group C, without supplementation, served as control.

Table 1. Experimental design

Animal	Period			Animal	Period			Animal	Period		
	1	2	3		1	2	3		1	2	3
1	A	B	C	4	B	C	A	7	C	A	B
2	A	C	B	5	B	A	C	8	C	B	A
3	A	C	B	6	B	A	C	9	C	B	A

Treatments: A = LSDairy, B = LS, C = control

Samplings and laboratory analysis

Samples of rumen fluid were taken 3 hours before and 3 hours after the morning feeding. Rumen pH, ammonia and VFA concentrations were measured and the redox-potential of the rumen fluid was determined, using the semi-quantitative method of the methylene-blue test.

PRELIMINARY RESULTS

Rumen fluid pH decreased in all groups after feeding (as expected) and no significant difference was found between them *(Figure 1.)*. Ammonia concentrations remained unchanged in the control group, significantly decreased in both groups receiving supplementation (*Figure 2*.). Difference between the two supplemented groups was not significant. According to the methylene-blue test results, the redox potential of rumen fluid samples were physiological both before, and after feeding, showing a slight decrease in reduction times after feeding (*no data shown*). No significant difference was found between groups. Total VFA-concentration (*Figure 3*.) increased after feeding in all groups. The degree of increase was significantly higher in Groups A and B compared to control. Changes in the molar proportion of VFAs are shown in (*Figures 4–6.*). There was no significant difference in the degree of increment between any of the groups.

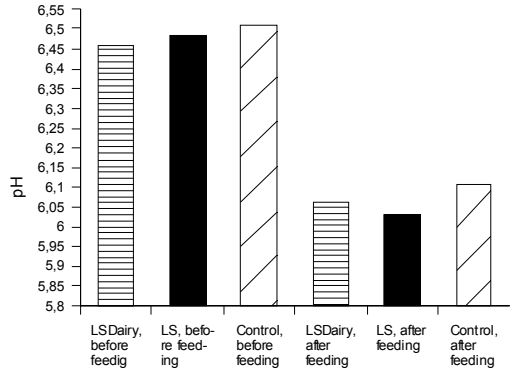

Figure 1. Rumen fluid pH

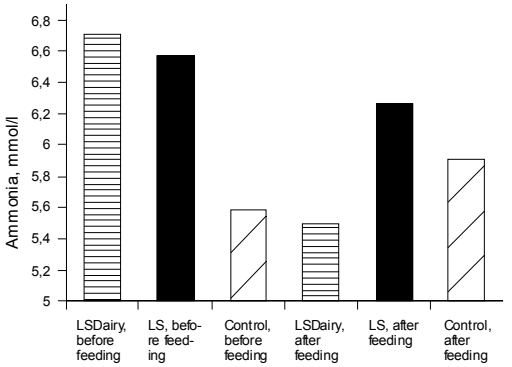

Figure 2. Ammonia concentration in rumen fluid

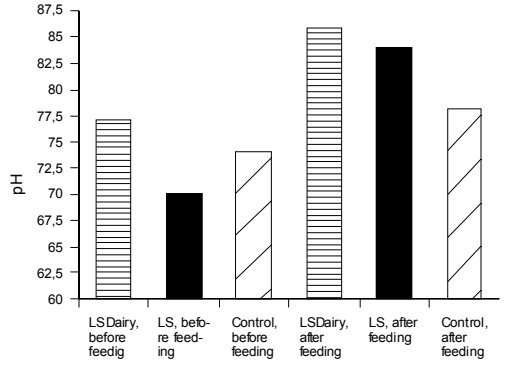

Figure 3. Total volatile fatty acid concentration in rumen fluid

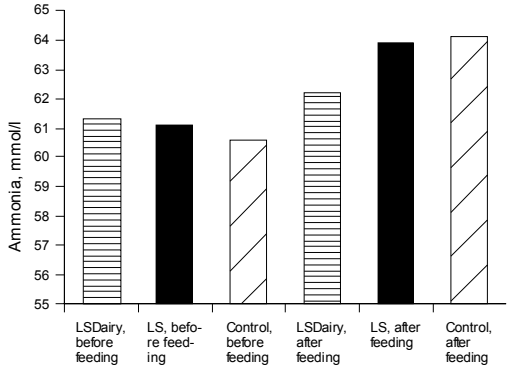

Figure 4. Molar proportion of acetate in rumen fluid

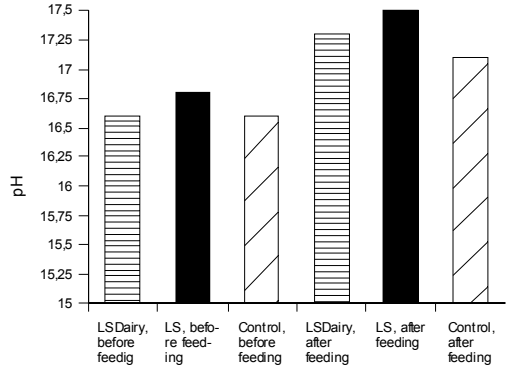

Figure 5. Molar proportion of propionate in rumen fluid

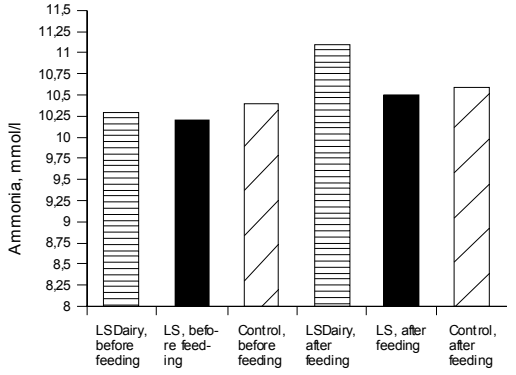

Figure 6. Molar proportion of n-butyrate in rumen fluid

UDDER FORMATION EXPERIMENT

Kleisiari, M.

Agamemnonos 4, 40200 Elassona, Greece; E- mai: mdbouzoukis@yahoo.com

The aim of this experiment is to see if different protein level in feeding sows during pregnancy has any influence on their milk production capacity. In practice as an index of milk production capacity of sows is the weight of suckled lot of piglets at 21 days after parturition.

At farrowing, one group of sows that received a deficient diet (11% protein level) during all gestation period can be compared with smaller groups of sows that received corrected diet (13% protein level) for different terms before parturition. After parturition, all sows in the experiment received a correct diet (16% protein level). In this way it is possible to appreciate effects of different terms of a low and of the normal protein level during pregnancy on the mean of bodyweight of piglets at the age three weeks and on the suckling capacity of sows.

I decided to use the same recommended diet for both lots of sows in order to avoid influence of a new nutritional difference between the corrected and the uncorrected feeding. Normally, I used a correct diet to give the opportunity to the different feeding during pregnancy to show its effect in the best possible way. At the same time this movement could allow to the sows with uncorrected feeding to react to a good feeding during lactation since 21 days of a new diet is enough a long term to show its effect. According to this point of view, all sows receiving the same diet for the same length of time must react in the same way.

Suckling capacity is indicated by the weight of the litter at the age of 3 weeks. The number of suckling piglets could influence the milk production capacity of sows having in mind that a greater number of piglets stimulate producing milk by more frequent sucking. On the other hand the mean weight of piglets in the suckling lots helps, more or less, to understand how the number of piglets is buffered by their individual weight.

In table 1 the number of sows per groups of increasing length of corrected protein level feeding, each time ten days more, and their suckling capacity are presented. Data concerning the milk production of sows are preceded by data indicating the number of piglets in each group at 21 days of age and the medium weight of piglets at that time. These indices are presented because each of them can influence or express, in some limits, the milk production ability of sows. A larger number of piglets stimulate milk secretion by more frequent sucking. Heavier piglets is due to higher sucked milk. Litter total weight combines merits of both these two indices in one synthetic index giving the opportunity to estimate better the suckling capacity of sows.

Table 1. Sows suckling capacity related to the type of feeding during pregnancy

Days of different feeding	Type of feeding	Mean number of sows in groups	Mean number of piglets in lots	Mean body weight of piglets	Mean suckling capacity of sows	Standard deviation of suckling capacity	**V %**
10	**C**	8	9.6	3.22	31.65	5.42	17.2
	D	8	9.4	3.29	30.96	6.78	21.9
20	**C**	6	10.5	3.19	33.50	6.89	20.6
	D	6	9.7	3.14	30.50	6.80	22.7
30	**C**	4	8,5	3.91	33.25	2.38	7.2
	D	4	9.2	2.91	26.75	5.00	18.7
40	**C**	3	7.7	4.38	33.75	3.82	11.3
	D	3	6.7	4.33	29.00	3.38	11.7
50	**C**	3	8.7	4,25	37.00	3.17	8.6
	D	3	9.3	3.50	32.60	3.36	10.3
60	**C**	8	11.9	3.41	40.60	4.56	11.2
	D	8	11.1	3.19	35.40	4.22	11.9
70	**C**	8	10.2	3.84	39.12	4.25	10.7
	D	8	10.0	3.26	32.63	6.09	18.7
80	**C**	8	10.1	4.11	41.50	3.16	7.6
	D	8	9.2	3.78	34.80	6.01	17.3
90	**C**	4	9.5	3.79	36.00	1.15	3.2
	D	4	9.1	3.24	29.50	3.51	11.9
100	**C**	5	7.8	4.81	37.50	3.83	10.2
	D	5	7.8	4.00	31.20	3.27	10.5

In this experiment, I used the same running as I have already mentioned in a previous experiment which mounted in a commercial reproduction pig farm where pregnant sows received an 11% level of protein diet. When the test started half of the pregnant sows received a corrected feed of 13% level of protein meanwhile the other half was fed further on the former diet. From this moment on, all sows that farrowed were fed on the same diet containing 16% of protein. Piglets were weighted 21 days after birth and the weight of the lot of suckling piglets was used as index for the milk production capacity of sows.

In order to judge how the protein level of diet during pregnancy acts on the udder formation for lactation the mean weight of piglet body weight were compared. Piglets were the progeny of two lots of sows with the same interval of time from the parturition and after in one of them feeding was corrected for protein content. Means were compared using the Student's *"t" test.*

Table 2. Significance of difference between suckling capacity of sows

Different feeding days	Type of feeding during pregnancy						Difference of means	D F	Calculated "*t*"	Tabulated "*t*" for 5%	Significance of difference
	Corrected (C)			Uncorrected (D)							
	No. of sows	Suckling capacity	s^2	No. of sows	Suckling capacity	s^2					
10	8	31.6	29.4	8	31.o	46.0	0.65	14	0.01	2.14	–
20	6	33.5	47.5	6	30.5	46.3	3.00	10	0.69	2.23	–
30	4	33.2	5.7	4	26.8	25.0	6.50	6	2.03	2.45	–
40	3	33.8	14.6	3	29.0	11.4	4.80	4	1.33	3.91	–
50	3	37.0	10.0	3	32.6	11.3	4.40	4	1.35	3.91	–
60	8	40.6	20.8	8	35.4	17.8	5.20	14	2.22	2.14	+
70	8	39.1	18.1	8	32.6	37.1	6.50	14	2.31	2.14	+
80	8	41.5	10.0	8	34.8	36.1	6.70	14	2.67	2.14	+
90	4	36.0	1.3	4	29.5	12.3	6.50	6	3.05	2.45	+
100	5	37.5	14.7	5	31.2	10.7	6.30	8	2.59	2.31	+

The table, containing the Student's "*t*" test counting, shows that the content of protein in the pregnant sow diet has effect on the productivity of the lactating sows. Probably a lower than 13% of protein content in the diet of pregnant sows do not permit a good formation of mammal tissue for lactation. It is interesting to notice that the proliferation process of mammal tissue requires more time than the fetus body mass increase. The recommended 13% of protein in pregnant sow diet has to be given to sows 60 days before parturition whilst for a normal growth of fetuses 30 days of this diet before birth seems to be enough. If that is true, I should find a significant difference between the mean suckling capacity of sows fed on corrected diet for 10 days and the one of sows fed on corrected diet for 60 days. In this case Student's "*t*" test shows:

$$\sigma^2 = \frac{8x29.4 + 8x20.8}{8+8-2} = \frac{235.2+166.4}{14} = 26.69 \qquad \sigma = \sqrt{26.69} = 5.166$$

$$\hat{\sigma} = 5.166x\sqrt{\frac{1}{8}+\frac{1}{8}} = 2.583 \; t = \frac{9}{2.583} = 3.48$$

So the value of "*t*" for 14 degrees of freedom is higher than 2.145 the value of "*t*" which indicates a probably significant level of difference. Really this value exceeds the 1% level of probability for a significant difference.

This not the case of the difference between suckling capacity of sows fed on corrected diet for 10 days and for 50 days. In this case:

$$\sigma^2 = \frac{8x29.4 + 3x10.0}{8+3-2} = \frac{235.2+30.0}{9} = \frac{265.2}{9} = 29.47 \; \sigma = \sqrt{29.47} = 5.43$$

$$\hat{\sigma} = 5.43\sqrt{\frac{1}{8}+\frac{1}{3}} = 5.43x0.67 = 4.11 \; t = \frac{5.4}{4.11} = 1.31$$

For 9 degrees of freedom this value of "*t*" shows that the difference of the two means is not significant. There is no doubt that the proliferation of mammal tissue for the next lactation requires at least 60 days of good feeding.

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INFLUENCE OF HALLOYSITE ADDITIVE IN HENS FEEDING ON BIOLOGICALLY ACTIVE EGG-WHITE COMPONENTS ACTIVITY

Kołacz, R.[1], Kulok, M.[1], Opaliński, S.[1], Teresa, S.[2]

Wrocław University of Environmental and Life Sciences; [1] Department of Animal Hygiene and Ichthyology; [2] Department of Animal Products Technology

SUMMARY

The use of natural and synthetic aluminosilicates in the animal production has been intensively researched in the recent years. They are characterised by selective absorption and high ion replaceable effect. They could also impact the digestive processes and metabolites binding in which decrease toxic gases emission from the litter without evoking negative changes in physiological parameters.

Research was carried out on lying hens. The aim of the research was to define the influence of halloysite on biologically active eggs' components. Haloysite was given with fodder to animals in 1 and 2%. Research of biological activity of egg-white components was carried out on eggs from each experimental group. The content of lisosyme, cystatine and antitrypsyne activity was marked. Feeding hens by fodder enriched by halloysite has significantly influenced the cystatyne content in egg-white of examined eggs increased also ability to inhibit trypsyne by ovomucoide and ovoinhibitor. Aluminosilicate additive to fodder didn't influence the lysozyme activity, which maintained the same level in all experimental groups.

Keywords: lying hens, aluminosilicates, halloysite, cystatyne, lysozyme, ovomucoide, ovoinhibitor

INTRODUCTION

The use of natural and synthetic aluminosilicates in the animal production has been intensively researched in the recent years. Natural and synthetic sorbents are porous, negatively charged material with sorptive properties depends on the particles' structure and polarisation degree and pore diameters. They have specific catalytic properties. They are also characterised by selective absorption and high ion replaceable effect. Halloysite, montmorillonite, vermiculite, pearlite, bentonite, zeolite, (heulandite and clinoptiolite) and synthetic HSCAS (Hydrated Sodium Calcium Aluminosilicate) are the names that are the most commonly found in the scientific communicates. These substances have sorptive properties towards heavy metals, some gases, mycotoxins and supplement the diet in trace elements. They could also impact the digestive processes and metabolites binding in which decrease toxic gases emission from the litter without evoking negative changes in physiological parameters.

Favourable effects of some aluminosilicates in animals' feeding were proved by many authors. (Kubena et al., 1991; Kyriakis et al., 2002; Papaioannou et al., 2002) In Poland a natural aluminosilicate is halloysite, which has rich deposits not explored before in the vicinity of Legnica. Determining how the addition of halloysite to laying hens' fodder influences health and

consumption safety of eggs will enable applying halloysite as additive to fodder. Hens' eggs are one of the best known products of „designed food" type. Food modifications consisting in putting halloysite (aluminosilicates) additive into fodder may influence the health of hens (Scheideler, 1993) and the components of eggs (Dobrzański et al., 1994). Those substances after isolation from the egg content are often applied as natural food preservatives or they determine durability of products consisting of egg-white.

Egg white contains biologically active components – lysozyme, cystatine, ovomucoid, ovoinhibitor and tripsine (Stevens, 1991**;** Guerin-Dubiard et al., 2006**)**. Lysozyme is an alkaline globular protein, which shows high enzymatic activity. Natural, biological lysozyme functions are aimed at protection of growing, which makes it an antiseptic substance. Moreover, lysozyme has the ability to inactivate viruses through bonding with its DNA, by creating inseparable complexes. As a substance of strong antibacteric and antivirus properties it finds broader and broader application in food industry as biopreservative and in pharmaceutical and cosmetic industry as natural antibiotic (Proctor et Cunningham, 1988). Cystatine shows also antiseptic and antiviral properties (Collins et al., 1998). It's an inhibitor of very strong effect with relation to ficyne and papaine. Furthermore, cystaine has the ability to inhibate cysteine proteinases like catepsine B, H and L (Saxena et Tayyab 1997).

Alterations in the cysteine proteinase inhibitor, cysteine proteinase ratios have been postulated to contribute to the malignant progression of tumours (Calkins et al., 1995). Especially important function of cystatine is connected with intra- and extracellular control of proteine decomposition, that's why it finds great interest in clinic.

The egg-white contains protein called ovomucoid that does not coagulate from solution by heating. There is 11% of ovomucoid in hens' egg-white and 15% in egg-white of ducks and geese. Ovomucoid from eggs of various birds' species can be various ensymes inhibitor, i.e. ovomucoide from hens' egg-white inhibits only trypsyne, but that from ducks or turkeys – trypsyne and chymotrypsyne.

Trypsyne and proteinases of bacterical and mouldical origin are inhibited by compound of protein nature called ovoinhibitor (Broadway, 1997). Ovoinhibitor can also disactivate chymotrypsine but differs from ovomucoide as regards working specificity. Inhibitors mentioned above possess very sophisticated and specific activity. Ovomucoide i ovoinhibitor inhibit the activity of serine proteinases and cystatine inhibites activity of tiolic proteinases. Cystatine i ovoinhibitor inhibit many proteolitic ensymes and ovomucoide only trypsyne (Saxena et Tayyab 1997). All mentioned inhibitors are highly thermostable (Acker et Ternes, 1994). In hens' egg-white their activity is well-known.

MATERIAL AND METHODS

Research was carried out on 60 hens (20 in one group), lasting 8 weeks. The aim of the research was to define the influence of halloysite on biologically active eggs' components. The experiment was conducted in controlled conditions in the animal vivary. Laying hens (ISA SHAVER) were kept in battery system (SPECHT). Provided microclimatic conditions were fitting standard norms established for laying hens. Animals were provided with permanent access to water. The fodder was passed every day at 8 a.m. in the amount of 125g/bird/day. Halloysite used in the experiment was raw (HSD) and activated in the sulphuric acid environment (HAV).

Laying hens were assigned to three groups. Before starting the experiment the animals had been given for 4 moths fodder as follows: control group – full-portion mixture Dolpasz „EXTRA

N-1/0 16%, experimental group I – full-porton mixture Dolpasz „EXTRA N-1/0 16% with 2% HAV addition and experimental group II – full-portion mixture Dolpasz „EXTRA N-1/0 16% with 2% HSD addition.

Research of biological activity of egg-white components was carried out on 30 eggs from each experimental group. The content of lysozyme, cystatine and antitrypsyne activity was marked.

During antitrypsyne activity of egg-white marking (Broadway, 1997) there was applied trypsyne reaction with synthetic substrate BapNA (N-benzoylo-DL arginino p-nitroanilide) as a result of which p-nitroanilin of yellow tint and maximum of absorbance by 412nm is secreted. The inhibitor's ability to inihibit trypsyne was examined by adding appropriate amount of inhibitor to samples, which resulted in drop of absorbance by 412 nm to control examination (without inhibitor).

Cystatine was marked by the test of cystatine to papaine inhibition activity (Siewiński, 1991). Antipapaine test is based on colorimetric marking of amount of freed hydrolyse BANA (chlorowodorek Na-benzoilo-DL-arginylo-B-naftylamidu) products as a result of cysteine proteinase (papaine) activity.

1 unit of inhibitor activity corresponds with 1 unit of papaine enzymatic activity (the amount of enzyme hydrolysing 1,0 mM of substrate per minute in standard conditions (37 degrees Celsius).

Lysozyme was marked with spectrophotometric method. The principle of marking consists in the measurement of dynamism of clouding changes in *Micrococcus lisodeicticus* suspension, which is exposed to incubation with appropriately diluted lysozyme. Measurement is conducted at a stable temperature of 25 degrees Celsius and the wavelength of &=450nm, specifying the fall of absorbance every 60 seconds during 6 minutes.

The results from the research were worked out statistically with the help of Statistica ver.7.0 computer programme. One-way variance analysis was carried out, averages were compared with the help of Duncan test. Differences at the level of $p \leq 0,05$ were considered as statistically significant.

In the study of group results the homogenous results were marked with the same letters, i.e. a, b, c.

RESULTS

Feeding hens by fodder enriched by halloysite has significantly influenced the cystatyne content in egg-white of examined eggs. There was observed twice and threefold increase of cystatine activity (4,62 units control) in the group with 2% HAV addition (12,8 units), and 7,41 units in case of HSD addition (Tab.1). Diverse feeding caused also increased ability to inhibit trypsyne by ovomucoide and ovoinhibitor. Antitrypsyne activity rose in egg-white of hens fed with fodder with additive of HAV up to 14,6 units and HSD up to 16,4 units in relation to control examination and came to 11,1 units per 0,1 mg protein. Aluminosilicate additive to fodder didn't influence the lysozyme activity, which maintained the same level in all experimental groups.

Probable reason of changes could be the fall of fodder contamination by bacteria, fungi and mycotoxines observed in fodder supplemented with halloysite, what has its effect on the health state (Kołacz, 2004).

CONCLUSIONS

1. Feeding laying hens on fodder enriched by halloysite caused twice and threefold twice increase of cystatine activity, which may show its influence on hens' health.
2. Halloysite additive to fodder influenced increasing ability of trypsyne inhibiting by ovomucoid i ovoinhibitor.

Table 1. Biologically active substances in egg-white

Experimental group	Biologically active substances		
	Cystatine [units/3mg of egg-white]	Lisosyme [units/1mg of egg-white]	Trypsyne [units/0,1mg of egg-white]
Control	4,62[a]	9,69[a]	11,1[a]
HAV	12,38[c]	10,01[a]	14,6[b]
HSD	7,41[b]	9,27[a]	16,4[c]

a,b,c – common letter in indexes of two averages indicates lack of statistically significant difference by P=0,05 (n=30)

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THE EFFECT OF LACTOPEROXIDASE SYSTEM ON ENHANCING THE MICROBIOLOGICAL QUALITY OF GOAT MILK AND YOGHURT

Magdoub, M.N.I.[1], El-Gendy, M.H. [2*], Kamel, Y.M.[2], El-Nawawy, M.A.[1]

[1] Food Science Department, Faculty of Agriculture, Ain Shams University, Cairo, Egypt;
[2] Animal Production Division, Desert Research Center, Ministry of Agriculture., Cairo, Egypt

SUMMARY

The objectives of this study were to determine the effect of activation of lactoperoxidase system (LPs) to increase the shelf life of goat milk and yoghurt. Samples of goats' milk were collected, taken under complete aseptic conditions, during December 2004 to May 2005 in North Sinai. These samples were divided to three combination groups from sodium thiocyanate and sodium percarbonate were tested for LPs activation as follows: Group 1 (G_1) (14 mg/L + 30 mg/L), Group 2 (G_2) (15 mg/L + 10 mg/L) and Group 3 (G_3) (20 mg/L + 25 mg/L), respectively. In general, Activation of LPs in goat milk caused a considerable slowing down rate of increase in titratable acidity during storage at 18–24 ^{o}C as compared to the control. The titratable acidity was increased from 0 until 72 hours in all groups; this effect was most pronounced in G1. The TABC was highly decreased significantly ($P < 0.01$) from 0 to 24 hours by 45, 40 and 26% in G1, G2, and G3, respectively. While, at 48 hours the TABC were increased by 19, 23 and 11% in G1, G 2 and G 3, respectively. This result could be occurred due to the LPs effect was decreased after 24 hours, which helping to increased TABC. The PC, SC and CC were in the same trend as TABC. The results of LPs application in yoghurt show that, the titratable acidity of yoghurt was not changed across the time from 1 to 21 days in both G1 and G2. While, in G3 was rapidly decreased after 7 days until 21 days. In G1 and G2 the TABC was decreased after 7 days of storage to 7.9 and 6 log cfu/ml, respectively. In contrast, at 14 days, the TABC was increased in G1 and G2 while, G3 was decreased in the same time. Moreover, at 21 days, the TABC was decreased in G1 and G2. The coliform was even more affected by LPs than the TABC. In all LPs groups, the yoghurt coliform was not detected throughout the different refrigeration periods.

Keywords: lactoperoxidase system, goat milk, yoghurt

Abbreviation key: LPs = lactoperoxidase system, **TABC** = Total Aerobic Bacterial Count, **PC** = Proteolytic bacterial Count, **SC**= Spore forming bacterial Count, **CC**= Coliform bacterial Count.

INTRODUCTION

The total number of goats in Egypt is about 5 millions heads. Average daily milk yield of goat in Egypt is varied and ranged from 0.2 to 1.2 kg /head/day according to different location, breeds and stage of lactation. Moreover, the average lactation period ranged from 120 to 180 days. They account for about 61% of the total number of the animals' population (MoALR, 2005). Milk is considered as the best environment to activate and grow of bacteria. Therefore it is subjected to contaminate by bacteria and (or) yeast. The major natural antimicrobial proteins of milk are

Lactoperoxidase system, Lysozyme, Lactoferrin and Immunoglobulins. The lactoperoxidase enzyme (EC 1.11.1.7) is present at concentration of 0.1–0.7 µg /ml in goat milk (Nadiu, 2000; Fonteh et. al. 2002). But the enzyme requires extra different concentrates of hydrogen peroxide and thiocyanate to activate it; in this case it is called lactoperoxidase system (LPs). The LPs has been recommended for preservation of raw milk in areas where it is not possible to use mechanical refrigeration for technical and/or economic reasons (IDF, 1988; FAO, 1999). The objectives of this study were to determine the effect of activation of lactoperoxidase (LPs) to increase the shelf life of goat milk and on the manufacture of yoghurt.

MATERIALS AND METHODS

Milk Samples: Samples of goats milk were collected form El-Arish city (North Sinai governorate) about 320 km North East of Cairo during December 2004 to May 2005. Data were collected as a part of the project sponsored by MERC, USA titled "Multinational approaches to enhance goat production in the Middle East". The milk was collected under complete aseptic conditions during the middle stage of lactation season and subjected individually to analysis by California mastitis test (CMT) to avoid the mastitis samples. The first three squirts of milk were discarded from each teat and samples were collected into sterile bottles and transmitted to the laboratory for bacteriological examination at 8°C.

Chemicals: **Sodium percarbonate ($Na_2Co_3.3H_2O_2$): was obtained from BDH chemicals Ltd. Poole England.**
Sodium thiocyanate (Na SCN): LOBA chemie PVT.LTD was used as a source of SCN^-.

Activation of Lactoperoxidase System: Sodium thiocyanate (Na SCN) and sodium percarbonate (Na_2CO_3. $3H_2O_2$) were used to activate LPs, three combination groups were tested. Group 1 (G_1) was 14mg/L Na SCN + 30mg/L $Na_2CO_3.3H_2O_2$, Group 2 was (G_2) 15mg/L Na SCN+ 10mg/L $Na_2CO_3.3H_2O_2$, and Group 3 was (G_3) 20mg/L Na SCN+ 25mg/L Na_2CO_3. $3H_2O_2$.

Yoghurt manufacture: Four Yoghurt groups were made (three made from three LPs treated groups plus control made from untreated goat's milk after heat treated at 90°C for 10 min. and cooled to 42°C). Starter (Freeze dried lactic culture for Direct Vat Set (DVS) Thermophilic lactic culture, type Yoghurt) was added at the rate of 2% and incubated at the same temperature. Later, yoghurt samples within each group were incubated at 42°C during 4–6 hours, until coagulation occur. These samples were transferred to a refrigerator and storage at 10°C. Microbiological analyses were done to measure acidity (AOAC, 1990) at 0, 7, 14 and 21 days.

Microbiological analysis

1. **goat milk:** According to American Public Health Association (APHA, 1992) the following bacteria were counted in different types of specific media for control and 3 LPs treated milk groups at 0, 24, 48 and 72 h.
   Total Aerobic Mesophilic Bacterial Count (TABC) was estimated using standard plate count agar medium,
   Proteolytic Bacterial Count (PC) was estimated using Standard plate count agar with 10% skim milk,

Spore forming bacterial count (SC) was estimated using Stander plate count agar with 0.1% soluble starch and

Coliforme bacterial counts (CC) were counted using Violet Red Bile Agar medium

2. **Yoghurt:** Yoghurt samples were prepared according to Tamime and Robinson (1999). Standard Plate Count Agar was used for enumeration to total bacterial count and Mac-Conkey broth was used for enumeration coliform bacterial count: (Most Probable Number (MPN)).

Statistical Analysis: Data of the two experimental were analyzed by the General Linear Model (GLM) procedure of SAS. (1998), according to the following model:

$Y_{ijk} = \mu + G_i + T_j + e_{ijk}$

Where,

Y_{ijk} = any observation,

μ = overall mean,

G_i = the effect of i^{th} LPs groups i =1–4,

T_j = the effect of j^{th} times j =1–4,

e_{ijk} = the residual assumed to be normally and independently distributed with mean 0 and variance σ^2_e.

RESULTS AND DISCUSSIONS

Effect of LPs on titratable acidity, pH and some flora: The results presented in Table 1 show that, there were significant ($p<0.05$) differences between the LPs groups and between times on all studied traits.

Table 1. Analysis of variances of the effects of using LPs group in goat milk at different times

S.O.V	df	*MS*				
		TA	TABC	PC	SC	CC
Total	79					
Time	3	0.09	8.54	3.31	9.12	5.91
Group	3	0.02	15.79	7.17	2.52	18.23
Error	73	0.01	3.42	3.68	5.96	6.59

S.O.V = source of variation, MS = means squares, df = degree of freedom,
TA = Titratable Acidity, TABC = Total Aerobic Bacterial Count,
PC = Proteolytic Count, SC= Spore forming bacterial Count,
CC= Coliform bacterial Count.

Titratable acidity % in goat milk after LPs activation: As can observed from results present in Table 2. Activation of LPs in goat milk caused a considerable slowing down rate of increase in titratable acidity during storage at 18–24 °C as compared to the control sample. These results were confirmed with the results obtained by Kamel and El Shaer (2004). They were estimated the acidity percentages as 0.13, 0.15, 0.17 and 0.18% in Shami goat milk in North Sinai of Egypt in the same level of LPs as G1 (14 mg/L Sodium thiocyanate plus 30 mg/L Sodium percarbonate) under different 4, 20, 30 and 40°C, respectively. These results were also confirmed the results obtained by Haddadin *et. al.* (1996), which estimated the acidity percentages as 0.16, 0.21 and

0.3% in Caprine goat milk under the same level of LPs as G2 (15 mg/L Sodium thiocyanate plus 10 mg/L Sodium percarbonate) at 4, 22 and 30 °C, respectively.

MICROBIOLOGICAL ANALYSIS OF GOAT MILK

Total Aerobic Bacterial Count (TABC) in LPs treated milk: Influence of LPs groups (Table 2) on TABC of goat milk stored at 18–24°C. That extraneous addition of Sodium thiocyanate and Sodium percarbonate immediate highly affected significantly ($p < 0.01$) on TABC for all treated groups as compared with control at 0 time. Result showed that, in control TABC was increased by about 11% from 0 to 24 hours and by the same percent from 24 to 48 hours. While, from 48 to 72 hours was decreased strongly by 20%. This result could be explained by the level of acidity was increased, which affected on TABC at 72 hours stored. While, in three groups the TABC was highly decreased significantly ($P < 0.01$) from 0 to 24 hours by 45, 40 and 26% in G1, G2, and G3, respectively. While, at 48 hours the bacteria were increased by 19, 23 and 11% in G1, G 2 and G 3, respectively. This result could be occurred due to the LPs effect was decreased after 24 hours, which helping to increased TABC. So, it could be recommended that, the LPs treated goat raw milk should not stored more than 24 hours at 18–24°C. In spite of that, in all studied groups the TABC was decrease at 72 hours due to acidity effect. Thus bacterial cells though multiplying might have been deprived of some metabolic function to spoil the milk.

Table 2. LSM ± SE of LPs groups and four different times in goat milk

Time (hours)	C		G1		G2		G 3	
	LSM	**±SE**	**LSM**	**±SE**	**LSM**	**±SE**	**LSM**	**±SE**
Acidity %								
0	0.16±0.044		0.11±0.044		0.12±0.044		0.12±0.044	
24	0.20±0.044		0.15±0.044		0.20±0.044		0.18±0.044	
48	0.29±0.044		0.21±0.044		0.29±0.044		0.28±0.044	
72	0.35±0.044		0.29±0.044		0.31±0.044		0.35±0.044	
Overall	**0.25±0.044**		**0.19±0.044**		**0.23±0.044**		**0.23±0.044**	
TABC (log/cfu/ml)								
0	5.92 ± 0.827		5.64 ± 0.827		5.87 ± 0.827		5.88 ± 0.827	
24	6.55 ± 0.827		3.90 ± 0.827		4.20 ± 0.827		4.66 ± 0.827	
48	7.26 ± 0.827		4.66 ± 0.827		5.15 ± 0.827		5.16 ± 0.827	
72	6.06 ± 0.827		3.55 ± 0.827		3.94 ± 0.827		4.07 ± 0.827	
Overall	**6.450± 0.827**		**4.44± 0.827**		**4.79 ± 0.827**		**4.94 ± 0.827**	
PC (log cfu/ml)								
0	4.81 ± 0.858		4.71 ± 0.858		4.84 ± 0.858		4.79 ± 0.858	
24	5.52 ± 0.858		4.23 ± 0.858		4.61 ± 0.858		4.89 ± 0.858	
48	6.96 ± 0.858		4.90 ± 0.858		5.34 ± 0.858		5.45 ± 0.858	
72	6.35 ± 0.858		4.13 ± 0.858		4.74 ± 0.858		5.45 ± 0.858	
Overall	**5.91± 0.858**		**4.49 ± 0.858**		**4.88 ± 0.858**		**5.15 ± 0.858**	
SC (log cfu/ml)								
0	2.74 ± 1.092		1.70 ± 1.092		2.33 ± 1.092		2.09 ± 1.092	
24	3.24 ± 1.092		1.78 ± 1.092		2.39 ± 1.092		2.66 ± 1.092	
48	2.31 ± 1.092		1.08 ± 1.092		1.13 ± 1.092		2.04 ± 1.092	
72	0.93 ± 1.092		1.50 ± 1.092		0.64 ± 1.092		0.87 ± 1.092	
Overall	**2.31± 1.092**		**1.15 ± 1.092**		**1.62 ± 1.092**		**1.91 ± 1.092**	

Table 2. Continuation

Time (hours)	C		G1		G2		G 3	
	LSM	**±SE**	**LSM**	**±SE**	**LSM**	**±SE**	**LSM**	**±SE**
CC (log cfu/ml)								
0	4.24 ± 1.148		3.82 ± 1.148		3.95 ±1.148		4.04 ± 1.148	
24	5.54 ± 1.148		2.27 ± 1.148		2.99 ± 1.148		4.16 ± 1.148	
48	6.23 ± 1.148		3.70 ± 1.148		4.43 ± 1.148		4.96 ± 1.148	
72	5.27 ± 1.148		2.61 ± 1.148		3.06 ± 1.148		3.56 ± 1.148	
Overall	**5.32 ± 1.148**		**3.10 ± 1.148**		**3.61 ± 1.148**		**4.18 ± 1.148**	

C = Control group.
G1 = GROUP1 (14 MG/L SODIUM THIOCYANATE + 30 MG/L SODIUM PERCARBONATE).
G2 = Group2 (15 mg/L Sodium thiocyanate + 10 mg/L Sodium percarbonate).
G3 = Group3 (20 mg/L Sodium thiocyanate + 25 mg/L Sodium percarbonate).

Proteolytic Bacterial Count (PC) in LPs treated milk: Table 2 show the effect of LPs on PC. PC is encountered in milk are quite resistant of LPs (Patel and Sannabhadti, 1993). PC in control group was increased by about 15% from 0 to 24 hours and by 26% from 24 to 48 hours. While, from 48 to 72 hours was decreased slowly by 9%. This result could be explained by the level of acidity was increased, which affected on PC in later stage of storage (72 hours). While, in all treated groups the PC was decreased significantly ($P < 0.01$) from 0 to 24 hours by 10, 5 and 2% in G1, G2, and G3, respectively. Moreover, at 48 hours the bacteria were increased by 16, 16 and 12% in G1, G2 and G3, respectively. This result could be occurred due to the effect of LPs was decreased after 24 hours, which helping to increased PC. So, it could be confirm the recommendation of the goat raw milk should not stored more than 24 hours after LPs treated at 18–24^{o}C. In spite of that, in all studied groups the PC was decrease at 72 hours due to acidity effect. Thus bacterial cells though multiplying might have been deprived of some metabolic function to spoil the milk.

Spore forming Bacterial Count (SC) in LPs treated milk: As can observe from results present in Table 2, in control group the SC was increased by about 18% from 0 to 24 hours. While, the SC was decreased by 29 and 60% at 48 hours and at 72 hours, in the three treated groups the LPs in goat milk samples caused a considerable slowing down rate of the SC during storage periods, as compared to the control. This effect was most pronounced in G1 in all treated groups. Moreover, in all treated groups the SC was increased significantly ($P < 0.01$) from 0 to 24 hr by 5, 2 and 27% in G1, G2 and G3, respectively. Then, at 48 hours the bacteria were decreased significantly by 40, 53 and 23% in G1, G2 and G3, respectively. In spite of that, in both of G2 and G 3 the SC was continuously decreased at 72h by 43, 57%, respectively, but in G1 the SC was increased by 40%.

Coliform Bacterial Count (CC) in LPs treated milk: Influence of LPs groups on the CC of goat milk stored at room temperature. It is evident Table 2. In control group, the CC was increased by about 31 and 13% at 24 and 48 hours, respectively. While, the CC was decreased by 15% at 72 hours. In both of G1 and G2, the CC was decreased significantly ($P < 0.01$) at 24 hours by 41 and 24%, respectively. While, in G3, the CC was increased by 3% at 48 hours. Moreover, the CC was decreased significantly ($P < 0.01$) at 48 hours by 63, 48 and 19% in G1, G2, and G3, respectively. In spite of that, in all studied groups the CC was decreased at 72 hours due to acidity effect.

Production of yoghurt from LPs activated goat milk: The results presented in Table 3 show that, there were significant ($p<0.05$) differences between the LPs groups and between times on all studied traits.

Table 3. Analysis of variances of the effects of using LPs group in yoghurt goat milk

S.O.V	df	*MS*		
		TABC	*CC*	**TA**
Total	16			
Time	3	1.54	1742500	0.02
Group	3	2.30	2102500	0.14
Error	9	1.72	1742500	0.02

P<0.01, df = degrees of freedoms, MS = Mean Squares. CC= Coliform bacterial Count.
TA = Titratable Acidity, TABC = Total Aerobic Bacterial Count,

Titratable acidity % of LPs treated yoghurt in goat milk: The results presented in Table 4 revealed that titratable acidity of yoghurt after 1 day from storage at 10 °C in G1 and G2 was 1.19 and 0.97% respectively. These values were less than 1.25% estimated in control sample and less than 1.35% obtained in G3. These results were the same as reported by Mehanna & Hefnawy (1988) and Nokuda *et. al.* (1996) which found that the Titratable acidity was decreased due to LPs activated in milk before manufactured the yoghurt. In general, the acidity was not changed across the time in both G1 and G2. This might be due to malfunction of starter culture as it is sensitive to antimicrobial agents. While, the acidity in G3 was rapidly decreased after 7 days until 21 days. This result shows that G3 with more effected on acidity than G1 and G2 due to different concentration of activated LP. These results imply that the addition of LPs suppressed the rate of acid production but has not effected on the bacterial growth which confirmed the same result obtained by Nokuda *et. al.* (1996).

MICROBIOLOGICAL ANALYSIS OF LPS TREATED GOAT MILK YOGHURT

TABC in LPs treated yogurt: As shows in Table 4, the TABC in yoghurt in G1 and G3 was higher than G 2 (refrigerated for 24 hours). The result showed that TABC in control groups was increased to 9.42 log cfu/ml after 14 days of storage. In G 1 and G2 the TABC was decreased after 7 days of storage to 7.98 and 6 log cfu/ml, respectively. While, in G3, the TABC was increased to 9.37 log cfu/ml. This result could be occurred due to the effect of LPs in TBAC within the different groups. In contrast, the TABC was increased in G1 and G2 after 14 days of storage, and G3 was decreased in the same time. While, the TABC was decreased in G1 and G2 after 21 days of storage. This result was confirmed the result obtained by Nokuda *et. al.* (1996), which the bacteriostatic effect was not exerted, but acid production was partially inhibited. This indicates that the starter cultures examined in this experiment showed varying degrees of sensitivity to the LPs, which confirmed the same result obtained by Seifu *et. al.* (2003). These results suggested storage time should not longer than 14 days in all treated groups accept in G3 which not longer than 7 day.

Coliform Bacterial Count in LPs treated yoghurt: As can be observed from results present in Table 4, the coliform was even more affected by LPs than the TABC with the effect begin most pronounced all treatments. The coliform continued to increase in the control samples through storage until 14 days. The coliform counts in control group in yoghurt was increased from 0 to 5400 MPN /ml then declined at 21days to reach 0 MPN /ml. However, in all LPs groups in yoghurt coliform was not detected throughout different storage times. This refer to the positive effect of LPs on the coliform bacteria

CONCLUSIONS

The LPs activation naturally increased the shelf life of milk to 48 hours after the system activated in goat milk at North Sinai of Egypt. The best LPs group, which naturally increases the shelf life of goat milk, was 14mg/L Sodium thiocyanate + 30mg/L Sodium percarbonate (G1). The best LPs group, which increases the shelf life of yoghurt, was 15mg/L Sodium thiocyanate + 10mg/L Sodium percarbonate (G2). The starter cultures showed varying degrees of sensitivity to the LPs. Group 3 showed more effect on acidity of yoghurt than G1 and G2. The LPs groups positively affect the TABC and CC of yoghurt. The yoghurt refrigeration time should not be longer than 14 days in all LPs treated groups except in case of G3 which not longer than 7 day.

Table 4. Average of LPs activated yoghurt in goat milk at different times

Storage Time (d)	Acidity %			
	C	**G1**	**G2**	**G3**
1	1.25	1.19	0.97	1.35
7	1.30	1.20	1.15	1.09
14	1.62	1.15	1.00	1.20
21	1.80	1.20	1.07	1.20
Overall	**1.49**	**1.19**	**1.05**	**1.21**
	TABC (log cfu/ml)			
1	7.67	9.36	7.40	9.12
7	8.62	7.98	6.00	9.37
14	9.42	8.94	8.93	6.00
21	8.06	7.41	5.00	7.70
overall	**8.45**	**8.42**	**6.83**	**8.05**
	Coliform bacterial count (MPN/ml)			
1	0	0	0	0
7	400	0	0	0
14	5400	0	0	0
21	0	0	0	0
overall	**1450**	**0**	**0**	**0**

C, G1, G2, and G3 as defied before

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WATER BORN GOITROGENS AND THEIR ROLE IN ENDEMIC GOITER AMONG LAMBS AND KIDS

Mottelib, A.A. and Mohamed, A.M.

Department of Animal Medicine, Faculty of Veterinary Medicine, Assiut University, Assiut, Egypt

ABSTRACT

The present work aimed to investigate the relationship between gross bacterial contamination of drinking water by human and animal sewage and the occurrence of goiter in lambs and kids. The study was carried out on 35 lambs and 67 kids with different clinical manifestations of goiter. Another group of apparently healthy animals was selected and used as a control for the study. Fifteen water samples were collected from different polluted watering sources of animals. The samples were subjected to bacteriological examination with special reference to pathogenic *Escherichia coli* strains. Blood samples were collected from all animals and were used for determination of thyroid function tests and some hemogram parameters. Tissue specimens for histopathological studies were collected from thyroid glands of clinical group after animals' slaughter. Pathogenic *E. coli* strains were detected in 10 water samples. Significant alteration was recorded in thyroid function parameters of clinically affected animals as compared to the control group. The anaemic manifestations reported in some diseased cases were confirmed by the reduction of studied blood parameters. Significant elevation in both total blood serum cholesterol and triglycerides were reported in all diseased cases. The thyroid glands of diseased lambs and kids showed histopathological abnormalities with focal lymphocytic infiltration. The study stress the role played by pathogenic *E. coil* strains as a goitrogenic agent for the disease and attracts the attention towards the potential danger of sewage pollution of drinking water sources and its impact on public health.

Keywords: small ruminants, goiter, *E. coli*, hormones, hemogram, lipogram

1. INTRODUCTION

The open sewage disposal in some water canals continue to threaten the public health in Egypt since many diseases can be transmitted by this practice. The rearing of animals, particularly sheep and goats on open areas, where such contaminated water canals being used for their watering, represent a potential danger for their health. Gross bacterial Contamination of drinking water by sewage is a cause of goiter in humans in countries where hygiene is poor (Radostits et al., 1994 and Bjergbæk and Roslev 2005).

The thyroid gland is the largest of the endocrine organs that function exclusively as endocrine gland. The functional unit of the gland is the thyroid follicle which is responsible for trapping, synthesis, storage, and release of thyroid hormones (Jones, et al., 1997). Conditioned iodine deficiency is directly related to the presence of goitrogens in foodstuffs offered to livestock. The thyroid gland is greatly affected by the nature of the diet and water, particularly goitrogenic substances Contained in them (Underwood, 1977).

The aim of the present work was to study the effect of sewage polluted water on thyroid gland function of lambs and kids. The study included the determination of the goitrogenic agents, namely *E.coli* as an indicator of sewage pollution, in drinking water and its relation to the clinic-pathological and histopathological alteration of thyroid gland and its function.

2. MATERIALS AND METHODS

1. Animals

A total of 35 lambs and 67 kids, 6–11 months old, were subjected to the study. The animals were collected from various flocks rearing by sewage-polluted water canals in some villages of Assiut. Based on clinical and clinic pathological findings, animals were subdivided into clinically and sub-clinically affected groups. A total of 18 healthy lambs and 12 healthy kids were collected from farms with clean water resources and were used as control groups.

2. Samples and adopted methods

2.1. Water samples: Fifteen water samples were collected from different sewage polluted waterways that constitute the main source for watering animals. The samples were evaluated for *E.coli* contamination using different polyvalent antiserum (Edwards and Ewing, 1972).

2.2. Blood: Two blood samples were collected from each animal, one with EDTA anticoagulant for haematological examination (Coles, 1986) and the other without anticoagulant for serum collection. Serum was used for the determination of thyroid stimulating hormone (TSH) (Bigos., 1984), Triiodothyronine (T_3) and Thyroxine (T_4) (Wood, 1980), Total cholesterol (Watson, 1960) and triglycerides (Royer, 1969).

2.3. histopathological examination: Thyroid gland tissue specimens were picked up from clinically affected group and were sent to histopathology laboratory, Faculty of Veterinary Medicine, Assiut University for histopathological evaluation according to (Jubb, et al., 1993).

2.4. Statistical analysis: Obtained data were statistically analyzed using one-way ANOVA (the findings were expressed as Means ± SE) by means of statistical Package for the Social Sciences for Windows (SPSS, version 10.0, Chicago, IL, USA),

3. RESULTS AND DISCUSSION

Out of the examined 15 water samples, 10 samples were positive for the typical pathogenic *E. coli* strains. Pathogenic strains of *E. coil* were determined using different polyvalent antiserum. This finding suggests a correlation between the presence of pathogenic *E. coli* strains and the occurrence of hypothyroidism in animals. This correlation can be explained in the light of the finding that pathogenic strains of *E. coli* synthesis anti-thyroid compound (Progoitrin), which diminishes the iodine uptake and inhibit organification by the thyroid gland (Gaitan, 1980).

Clinically affected animals included 10 lambs and 25 kids. The Prominent signs reported among clinically diseased lambs and kids were enlargement of the thyroid gland, the gland can be palpated if not visible in some cases. In General, diseased animals showed partial or complete loss

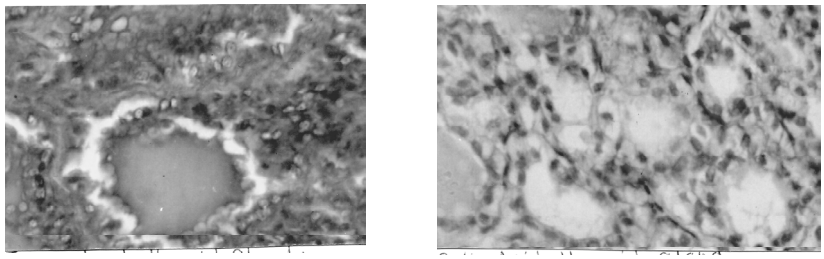
of their coat (hair or wool), extensive alopecia and weakness, murmurs and thrills were recorded at the jugular furrow of most diseased cases. The recorded clinical signs were more or less in harmony with Radostits et al., (1994). Skin pigmentation in some diseased cases could be a sequel of increased melanocytes in epidermis as a response to hypothyroidism (McDonald and Pineda, 1989). Murmur and thyroid thrill were due to the increased arterial blood supply of the gland. Gupta, et al (1998) added that hypothyroidism has a negative effect on growth.

The thyroid function parameters of the clinically-affected groups have showed highly significant deviation ($P<0.001$) as compared to the control group. The rest of the examined animals, 25 lambs and 42 kids, comprised the sub-clinical groups that did not show apparent clinical manifestation of goiter, however, their thyroid function parameters were significantly deviated ($P<0.05$) from that of the control groups (Table 1). Similar results were recorded by Sokkar et al, (2000). However, TSH has showed contrary pattern of $T_3$and T_4 as it showed significant increase. Elevated TSH explained the enlargement of thyroid gland (McDonald and Pineda 1989). The increased values of lipid components were the reflection of the reduction of thyroid hormones (Feling et al, 1981). The lipogram picture of studies animals have showed significant elevation of total cholesterol and triglycerides in clinically affected groups. However, non significant changes were recorded in sub-clinical groups (Table 2). Haematological examination revealed non significant changes in both clinical and sub-clinical groups (Table 3). The reduction in the values of hemogram parameters was in accordance to that previously reported by Sokkar et al, 2000 in lambs and confirmed the anaemic manifestation recorded in diseased animals. These changes could be attributed to the lowered basal metabolic rate due to hypothyroidism.

Histopathological findings revealed marked pathological affection of thyroid glands of clinically affected animals. The gland has irregular follicles with tip nucleus and vacuolated cytoplasm of their lining epithelium. The lumen of the follicle was filled with vacuolated colloid. Inter-follicular inflammatory cell infiltration, increase vascularity and connective tissue proliferation were also recorded (Figure 1). The histopathological findings were in correlation with the thyroid gland functions of the clinically affected animals and were in accordance with that reported by Sokkar et al, (2000). Presence of lymphocytic infiltration supports the suggestion of secondary hypothyroidism due to *E. coli* infection.

4. CONCLUSIONS

The study attracts the attention for the danger of human and animal sewage that pollutes the environment as well as drinking water resources with the subsequent potential hazards on the general public health. The work stress the role of pathogenic *E. coli* strains as goitrogenic agent predispose for the occurrence of goiter in animals with uncontrolled drinking water sources.



(A) (B)

Figure 1. Histopathological finding of thyroid gland cross section showing: (A), Lamb thyroid gland with detached follicular epithelium, vacuolated colloid, congested blood vessels and interfollicular inflammatory cell infiltration (B), Kid thyroid gland with degenerated follicles infiltrated with inflammatory cells and fibrosis and follicles without colloid

Table 1. Mean Blood serum values ± SE of TSH, T3 and T4 in studied animals

Animal Groups		No.	TSH (µIU/ dl)	T3 (ng/dl)	T4 (µg /dl)
Lambs	Control group	18	2.89 ± 0.02	102.42 ± 3.07	5.31 ± 0.42
	Clinical group	10	6.18 ± 0.92***	48.25 ± 3.81***	1.81 ± 0.01***
	Sub-clinical group	25	3.31 ± 0.51*	88.43 ± 3.11**	4.01 ± 0.21*
Kids	Control group	12	3.12 ± 0.42	154.14 ± 3.92	4.12 ± 0.03
	Clinical group	25	7.11 ± 0.38***	72.35 ± 4.11***	2.21 ± 0.01***
	Sub-clinical group	42	4.18 ± 0.71*	118.71 ± 3.52**	3.88 ± 0.24*

Table 2. Mean values ± SE of some blood serum lipids in studied animals

Animal Groups		No.	Total cholesterol (mg/dl)	Triglycerides (mg/dl)
Lambs	Control group	18	76.2 ± 4.82	28.4 ± 1.51
	Clinical group	10	87.2 ±7.02**	46.7 ± 2.72***
	Sub-clinical group	25	78.5 ± 4.56	37. 2 ± 3.41*
Kids	Control group	12	110.8 ± 3.51	25.5 ± 1.32
	Clinical group	25	140.8 ± 3.11**	44.6 ± 2.05**
	Sub-clinical group	42	134.11 ± 4.81*	38.5 ± 3.31*

Table 3. Mean values ± SE of hemogram parameters in studied animals

Animal Groups		No.	RBCs (million)	PCV %	Hb gm %
Lambs	Control group	18	10.91 ± 0.21	38.81 ± 1.21	11.51 ± 0.81
	Clinical group	10	8.52 ± 0.91**	33.41 ± 1.02**	9.24± 0.02**
	Sub-clinical group	25	8.68 ± 0.94**	33.82 ± 1.08**	9.71 ± 0.87**
Kids	Control group	12	11.23 ± 0.35	32.61 ± 1.12	10.25 ± 0.49
	Clinical group	25	9.31 ± 0.67**	30.12 ± 1.15*	9.13 ± 0.18*
	Sub-clinical group	42	9.95 ± 0.94**	30.81 ± 1.09*	9.52± 0.41*

***(P<0.001), **(P<0.01), *(P<0.05)

5. ACKNOWLEDGMENT

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EVALUATION OF DRINKING WATER SOURCE IN DEPENDENCE ON SEASON AND GROUNDWATER QUALITY

Ondrašovič, M., Vargová, M., Ondrašovičová, O., Hromada, R., Venglovský, J., Sasáková, N., Laktičová, K. and Kudríková, D.

University of Veterinary Medicine, Košice, The Slovak Republic

INTRODUCTION

Quality of drinking water depends on location of the source and pollution of the respective region originating from industrial but also agricultural production. One can assume that both quantitative parameters, i.e. capacity of the source, and qualitative properties are affected also by climatic conditions, particularly temperature and precipitations, which vary throughout the year. The submitted study investigates the relationship between results of chemical and microbiological examination in relation to respective climatic conditions and the quality of ground water.

MATERIAL AND METHODS

Material

The investigated water source was located at a distance of 820 m from a village and approximately 185 m above its location Geomorphologically, the relief of the location can be characterised as hilly. Climatic-geographical type of the area is hollow basin with moderately warm climate. The soil type is sabulous clay.

Methods

Microbiological examination

The microbiological examination included determination of colony counts at 22 °C and 37 °C and coliform bacteria in accordance with the STATUTORY ORDER of SR No. 354/2006 and methods by ŠTĚPÁNEK et al. (1982) and HOLODA et al. (2006).

Chemical examination

Of the chemical parameters of drinking water quality we determined pH, ammonium ions, nitrates, chlorides, phosphates, free chlorine and COD_{Mn} according to respective ISO standards, HORÁKOVÁ et al. (1986) and by HACH (1992).

Statistical evaluation of results

We calculated parameters of regression line on the basis of normal equations. The evaluation concerned the potential influence of temperature and precipitation on the plate counts of selected micro-organisms. It was logical to assume that the plate counts (CFU) depend on the variable Y. The regression line equation assumes general form $y = a + b.x$. According to this equation we can, on the basis of empiric values of X, estimate the values of Y. Thus in our case, using the real

values of temperature or precipitations (X), we could estimate the theoretical level of plate counts CFU.

The scale of relationship tightness according to determination coefficient (R^2), expressed in per cent and read from the scale by GROFÍK et al. (1987) is (at small rounding) as follows: $R^2 < 10\%$ low relationship, $10\% \leq R^2 < 25\%$ moderate relationship, $25\% \leq R^2 < 50\%$ considerable relationship, $50\% \leq R^2 < 80\%$ tight relationship, $80\% \leq R^2$ very tight relationship.

The coefficient of determination expresses relative proportion of the influence of independent variable X on dependent variable Y (POLAČEK M., 1996).

RESULTS AND DISCUSSION

The water source was constructed for the purpose of supplying an Agrotourism centre with drinking water. In the beginning it was used for construction purposes which guaranteed preliminary clean up and adjustment of the internal space of the well. However, the results of subsequent microbiological and some chemical examinations failed to comply with the requirements on drinking water.

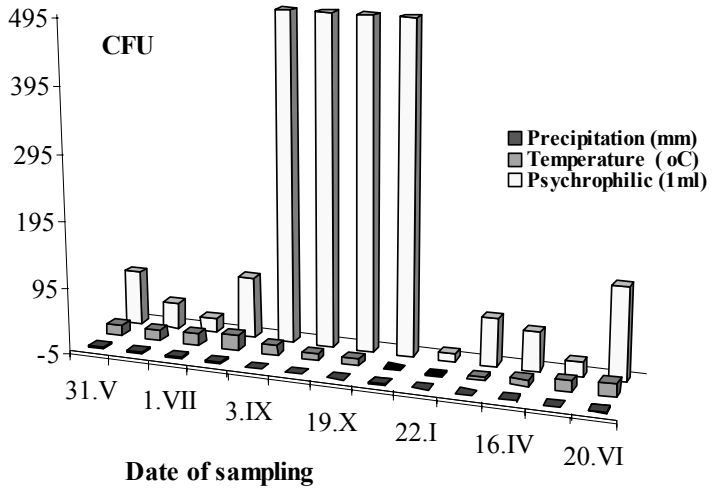

Figure 1. Plate counts of psychrophilic micro-organisms (CFU_{22}) at microbiological examination of drinking water source between May 31, 2001, and June 20, 2002

Figure 1 shows plate counts of psychrophilic micro-organisms (CFU_{22}) determined between May 31, 2001, and June 20, 2002. Psychrophilic plate counts reflect general contamination of water (ONDRAŠOVIČOVÁ, 2005). Maximum acceptable level of these bacteria in drinking water intended for mass consumption is 200 $CFU.ml^{-1}$ (STATUTORY ORDER No 354/2006). Fig. 1 indicates that between September and November 2001, when 4 samples were examined, the limit was exceeded considerably and reached even 500 CFU in 1 ml of water.

When evaluation the two additional groups of micro-organisms, namely mesophilic and coliform we also observed that their plate counts were increased similar to psychrophilicplate counts (Tab. 1).

Table 1. Plate counts of mesophilic micro-organisms (CFU_{37}) at microbiological examination of drinking water source between May 31, 2001, and June 20, 2002

	May 31	June 26	July 1	July 13	Sept. 3	Sept. 30	Oct. 19	Nov. 27	Jan. 22	March 12	April 16	May 6	June 20
Precipitations mm)	2.2	1.9	2	2.6	0.3	0.4	0	0.8	0.7	0.2	2.1	0.3	1.3
Temperature (^oC)	16.1	15.9	18.3	22.3	14.1	9.2	9.6	–0.8	–3.1	4.8	9.8	16.1	20.4
Mesophilic (1ml)	52	36	18	114	58	58	116	111	4	23	13	7	48

The requirement on plate counts of total coliforms (Tab. 2) concerning drinking water intended for mass consumption (absence of coliforms in 100 ml of examined water) was met only in one case. The examined source failed to meet even the less strict requirement on water for individual consumption (absence of coliforms in 10 ml of water) (ONDRAŠOVIČ et al., 1996).

Table 2. Plate counts of total coliforms at microbiological examination of drinking water source between May 31, 2001, and June 20, 2002

	May 31	June 26	July 1	July 13	Sept. 3	Sept. 30	Oct. 19	Nov. 27	Jan. 22	March 12	April 16	May 6	June 20
Precipitations (mm)	2.2	1.9	2	2.6	0.3	0.4	0	0.8	0.7	0.2	2.1	0.3	13
Temperature (^oC)	16.1	15.9	18.3	22.3	14.1	9.2	9.6	–0.8	–3.1	4.8	9.8	16.1	20.4
Total coliforms (1ml)	98	62	12	21	25	57	50	48	0	6	28	5	23

Figure 2 shows statistical evaluation of plate counts of psychrophilic bacteria in dependence on environmental temperature by means of the coefficient of determination which for psychrophilic micro-organisms corresponded to R^2 0.0693, or 6.9%, i.e. low relationship to temperature. Similar results were obtained for mesophilic micro-organisms, with R^2 equal to 0.0042, and for coliforms with R^2 equal to 0.01141.

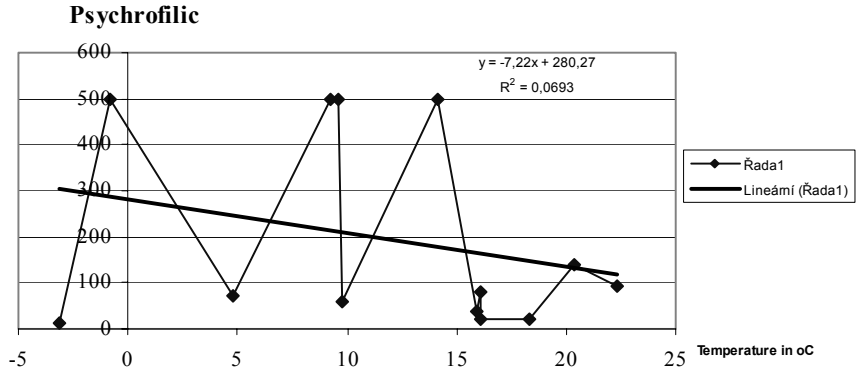

Figure 2. Relationship between temperature and plate counts of investigated groups of micro-organisms

On the basis of presented results and their subsequent evaluation which confirmed contamination of the water source, we carried out examination of water quality in the location taking all samples on the same day (July 27, 2002). In addition to village Varhaňovce, which served for comparison, we examined additional 11 water sources by examining in respective villages individual water sources (wells) and also water from public water main supplying the village (Tab. 3). The aim of this stage was to evaluate quality of ground water in the respective location. The results obtained (high plate counts of micro-organisms) point to contamination of ground water.

Table 3. Plate counts of investigated groups of micro-organisms in water samples from sources in selected villages

Place of sampling	Source	Psychrophilic (1 ml)	Mezophilic (1 ml)	Coliforms (10 ml)
Varhaňovce	Well	237	119	285
	Water main	117	55	64
Brestov	Well	NC	NC	NC
	Well	295	184	NC
	Water main	0	5	12
Bunetice	Well	NC	405	NC
	Well	NC	279	NC
Ortaše	Well	65	16	260
Šarišské Bohdanovce	Well	NC	396	NC
	Well	138	196	300
Vtáčkovce	Well	NC	NC	NC
	Well	300	360	144
	Stream	455	254	NC

NC – uncountable

The presence of ammonium ions in water is considered a proof of fresh decomposition processes. They are harmful due to their direct effect on organism and also serve as indicators of immediate pollution of water with organic substances including urea, faeces and similar, which increases risk to organisms. Higher concentrations of NH_4^+ in ground water may result from chemical and biochemical reduction of nitrates if they are of organic origin (ĎURIČKO, 2000). The maximum acceptable level in drinking water (0.5 $mg.l^{-1}NH_4^+$) was exceeded in all samples which is indicated by mean value of this parameter reaching 0.9 $mg.l^{-1}NH_4^+$ (Tab. 4). Determination of the level of NO_3^- showed that the maximum value detected was 23.0 $mg.l^{-1}$ which is below the maximum acceptable limit 50 $mg.l^{-1}NO_3^-$. Results of chemical oxygen demand only in 3 out of 13 cases corresponded to the requirement, i.e. were below 3 $mg.l^{-1}$. According to the findings for Cl_2, water from public water main in Brestov was disinfected which was also reflected in microbiological findings for this particular water source (Tab. 3).

Table 4. Results of chemical examination of sources of drinking water in the investigated location ($mg.l^{-1}$)

Place of sampling	Source	pH	NH_4^+	NO_3^-	Cl^-	PO_4^{3-}	Cl_2	COD_{Mn}
Varhaňovce	Well	6.4	0.83	7.0	111.1	0.22	0	3.4
	Water main	65	0.81	5.3	11.9	0.19	0	2.9
Brestov	Well	6.1	0.81	10.0	23.8	1.84	0	3.1
	Well	6.1	0.88	9.9	24.8	2.06	0	4.3
	Water main	6.5	0.77	3.6	7.5	0.24	0.29	2.4
Bunetice	Well	6.2	0.89	23.0	14.3	0.34	0	3.2
	Well	6.1	0.86	3.0	35.7	1.50	0	4.8
Ortaše	Well	6.7	0.68	5.3	44.7	0.03	0	3.1
Šarišské	Well	6.9	0.75	19.0	184.6	0.11	0	3.9
Bohdanovce	Well	6.7	0.84	2.3	198.5	0.21	0	3.9
Vtáčkovce	Well	6.4	1.71	4.5	5.6	0.21	0	2.4
	Well	6.2	0.94	6.1	151.8	0.11	0	3.1
Range		6.1– 6.9	0.68–1.71	2.3–23	5.9–198.5	0.03–2.06	–	2.4–4.8
Mean		6.4	0.90	8.25	67.8	0.59	–	3.4
Vtáčkovce	Stream	6.4	0.79	7.4	35.7	0.11	0	4.0

CONCLUSION

Results of examination of water in the village Varhaňovce showed that despite minimum possibility of contamination of the source from the outer environment with regard to its location, water from this source did not comply with the basic chemical and microbiological requirement on drinking water. Statistical analysis performed by means of the coefficient of determination indicated that environmental temperature had minimum effect on microbiological findings when considering examination of 13 water sources.

Results of examination of additional 12 water sources in the location from the microbiological and chemical point of view point to pollution of ground water in the respective area.

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EFFECT OF A MULTI-MICROELEMENT PREPARATION ON THE PRODUCTION AND METABOLISM OF BROILER RABBITS

Papp, Z. and Tegzes, L.

Szent István University, Faculty of Veterinary Science, Department of Animal Hygiene, Herd Health and Veterinary Ethology, István utca 2., H-1078 Budapest, Hungary; papp.zoltan@aotk.szie.hu

SUMMARY

Laboratory model experiments were conducted to test the effects of a multi-microelement preparation (Cuni-Stibol®) on certain production-, haematological and metabolic parameters. After weaning at 28 days of age New-Zealand White rabbits were randomly assorted into two control (untreated and positive) and four experimental groups. The groups were kept for 49 days in cages with optimum environmental condition and fed ad libitum with pelleted rabbit feed that contained coccidiostatic. Water was constantly available through nipple drinkers. Control rabbits consumed plain water. The water of the positive control group and experimental groups (3–6) was acidified with 7 ml 10% acetic acid per litre. The water of the four experimental groups was added a multi-microelement at 0.35, 0.7, 1.4 and 2.8 ml/litre concentration, respectively. The micro-element preparation did not have unpleasant organoleptic characteristics and improved weight gain and feed conversion rate. The preparation at 0.28% concentration had the best effect on FCR and total weight gain. The preparation did not have adverse effect on animal health.

Keywords: broiler rabbits, micro-elements

INTRODUCTION

The aim of our study was to test the effects of a multi-microelement preparation specially developed for rabbits (*Cuni-Stibol* produced by Béres Sc.) on certain production and metabolic parameters. The water soluble preparation contains chelated compounds of essential micro elements, even less-known ones as B, Ni, Se and V. Determining the micro- and trace-element demand of rabbits is a complicated, being caecotroph and rather costly field of research, thus testing the production- and health-related effects of such preparation seemed interesting.

MATERIALS AND METHODS

Animals and diets

After weaning at 28 days of age, New Zealand White rabbits were randomly assorted into two (untreated and positive) control (1,2) and four experimental (3–6) groups of 18. The animals were kept in the climate laboratory of the Faculty, providing optimal environmental and housing (3 animals/cage) conditions. During the 49 days of the experiment the animals were fed ad libitum with pelleted rabbit feed containing coccidiostatic and 5% complete premix (1517 ppm Fe; 111

ppm Cu; 320 ppm Mn; 1077 ppm Zn; 51 ppm I; 15,5 ppm Co). Water was constantly available from nipple drinkers. Dosage of the preparation was given in function with bodyweight, therefore a 72-hour pre-experiment was conducted in order to measure the daily water consumption of the animals. Based on the results (86 ml/rabbit; BW: 770–820g), the optimal concentration of the preparation was determined at 0,7 ml/litre. The water of the positive control and the experimental groups was acidified with 7 ml 10% acetic acid per litre to stabilize the preparation. The water of the experiment groups was supplemented with the preparation at 0.35, 0.7, 01.4, 2.8 ml/litre concentration, respectively. Micro-element concentration of the preparation was the following

Sampling and laboratory analysis

Feed intake was recorded daily, water intake was measured twice a week, and body weight gain was checked once a week. The health status was permanently monitored, dead and animals were culled from the statistical analysis, however. Mixed arterious and venous blood samples were taken once, under the anaesthesia preceding exsanguination and pathological examination, when the 49-day experimental period was over.

To monitor possible haematological changes, Hb-concentration and haematocrit value of whole blood were determined. Blood glucose-level, plasma AST activity, FFA-, triglycerid- total protein and urea concentrations were also measured to check metabolic status.

RESULTS AND DISCUSSION

Major production parameters are shown in *Table 1*. Feed intake did not differ significantly between groups. The water intake did not differ significantly. Total weight gain was highest in Group 5 and 6, though only Group 6 differed significantly ($P<0.01$) from untreated and positive control groups. Acidification had no effect on production. In function with feed intake and weight gain, the feed conversion rate of Group 6 significantly improved. Based on death records there was no connection between treatment and general health status of the animals.

Haematological and metabolic parameters are displayed in *Table 2*. Hb-concentrations were physiological in all groups, results in Group 6 were significantly higher than in the untreated control Group 1 ($P<0.05$). Haematocrit values did not differ significantly between groups. Blood glucose-levels showed no statistically significant difference. Plasma FFA-concentrations in Group 3 and 4 were significantly lower than that of the untreated control Group 1. Plasma triglycerid concentrations in Group 2 were significantly lower than in control Group 1. AST activity of blood plasma tended to be lower in experimental groups, though the difference was significant only in case of Group 3 and 5. No significant difference in total-protein and urea concentrations was found between groups.

According to the results of our study it can be concluded that the preparation does not have unpleasant organoleptic effects, when mixed in drinking water. It can be stated that supplementing drinking water with the preparation at 0.28% concentration significantly improves weight gain and feed conversion rate. All haematological and metabolic results were in the physiological range, thus it can be concluded that the preparation had no adverse effect on animal health.

Table 1. The effect of CUNI-STIBOL® on major production parameters

Parameters	**Groups**					
	Control	**2.**	**3.**	**4.**	**5.**	**6.**
Live weight at start, g	971 +/–130,5	959 +/–128,9	984 +/–140,7	960 +/–101,4	1006 +/–100,7	971 +/–102,4
Live weight at conclusion, g	2434 +/–284,6	2442 +/–308,7	2524 +/–279,5	2525 +/–214,2	2624 +/–285,4	2665* +/–201,7
Total weight gain, g/49day*rabbit	1462,7 +/–218,3	1482,7 +/–276,7	1540,0 +/–212,7	1565,0 +/–215,1	1617,5 +/–257,1	1694,7** +/–157,0
Daily weight gain, g/day*rabbit	29,9 +/–4,5	30,3 +/–5,7	31,4 +/–4,4	31,9 +/–4,4	33,0 +/–5,2	34,6** +/–3,2
Daily feed intake, g/day*rabbit	110,5 +/–13,3	110,4 +/–15,1	107,3 +/–12,8	113,0 +/–14,4	116,6 +/–13,4	114,3 +/–12,5
FCR, kg/kg	3,7	3,65	3,41	3,54	3,52	3,3
Water consumption, l/kg feed DM	1,93	2,07	1,93	1,93	1,76	1,88
Micro-element consumption, ml/49 day*rabbit	–	–	3,04	6,42	12,07	25,3
Micro-element consumption, µl/bwt kg*day	–	–	35,4	75,2	135,7	284,1

*, **: difference is statistically significant compared to control (P>0.05, P>0.01)

Table 2. The results of measuring the blood samples

	Groups					
Parameters	**Control**	**2.**	**3.**	**4.**	**5.**	**6.**
Hb (mmol/l)	7,65 +/–0,39	8,32 +/–0,92	8,07 +/–0,46	7,96 +/–0,61	8,17 +/–0,81	8,48* +/–0,61
Htc (l/l)	0,4 +/–0,025	0,42 +/–0,028	0,4 +/–0,011	0,41 +/–0,031	0,39 +/–0,024	0,04 +/–0,02
Glucose (mmol/l)	4,5 +/–2,55	5,38 +/–1,75	5,57 +/–0,69	4,76 +/–0,84	4,46 +/–1,17	6,07 +/–2,03
FFA (mmol/l)	0,240 +/–0,107	0,19 +/–0,111	0,144* +/–0,08	0,106** +/–0,052	0,264 +/–0,183	0,170 +/–0,05
Triglicerid (mmol/l)	1,25 +/–0,85	0,31* +/–0,06	1,0 +/–0,43	0,93 +/–0,24	1,38 +/–0,69	1,19 +/–0,61
AST (U/l)	34 +/–13	33,0 +/–8,0	22,0* +/–9,0	29,0 +/–7,0	24,0* +/–7,0	30,0 +/–10,0
Total protein (g/l)	70,0 +/–5,0	67,0 +/–6,0	70,0 +/–3,0	70,0 +/–5,0	69,0 +/–4,4	70,0 +/–2,5
Urea (mmol/l)	7,76 +/–1,03	8,27 +/–0,99	7,79 0,73	8,27 0,74	8,21 +/–0,77	7,57 +/–0,77

*, **: difference is statistically significant compared to control (P>0.05, P>0.01)

THE CUMULATION OF SELECTED CHEMICAL ELEMENTS OF TOXIC PROPERTIES IN BEE HONEY ORIGINATING FROM THE INDUSTRIAL AND RURAL-FOREST AREAS

Roman, A., Bartkowiak, A. and Reginia, M.

Wroclaw University of Environmental and Life Sciences, Department of Animal Hygiene and Ichthyology, Chełmońskiego 38 C, 51–630 Wrocław, Poland

SUMMARY

The aim of the investigation was determining accumulation level of selected chemical elements of toxic properties (Pb, Cu, Cd, Zn), in bee honey coming from the industrial and rural-forest areas. In honey samples there detected different levels average concentration values of the examined elements in honey originating from copper industry region were low and they amounted as follows: copper – 0,484, cadmium – 0,059, zinc – 3,343 and lead – 0,059 $mg \cdot kg^{-1}$ d.m. The samples coming from rural-forest region showed higher copper concentration – 1,353, lead – 0,147 and cadmium – 0,373 $mg \cdot kg^{-1}$ d.m., while zinc concentration was lower – 3,179 $mg \cdot kg^{-1}$ d.m. Statistically significant differences (at $P_{0,05}$) in copper and lead concentration there were recorded between the areas subjected to examination. In honey samples from both areas there were obtained significant (at $P_{0,01}$) correlations between cadmium and lead concentration level. Similarly significant (at $P_{0,05}$) correlations were proved between copper and cadmium concentration in honey collected from Legnica and Głogów copper district and between copper and zinc concentration in honey originating from the control region.

Keywords: honeybee, bee's honey, heavy metals, toxic elements

INTRODUCTION

Bee's honey is a product made by a honeybee from flower nectar or honeydew. Obtained from beehives, it constitutes a final product, without aim necessity of its technological processing. Yet, the raw materials from honey production brought by bees contain contamination coming from different sources. Therefore, bees and their product have been used to estimate the degree of environmental pollution through determination of heave metals concentration and other chemical elements of toxic properties [Muszyńska, 1995, Topolska et al. 2004]. In this way there are obtained the data regarding average contamination of particular area included within the range of bee flying, I.E. about 20 km^2, which is equal approximately 2,5 km from a beehive [Muszyńska, 1997]. Legnica and Głogów copper district is situated in Silesia Lower province and it covers an area of about 2200 km^2. Yearly output of copper ores amounts about 26 mln tons. Copper industry came into being in an agricultural region and in the vicinity of urban agglomerations, which compelled introduction of proecological undertakings. Main sources of pollution emission to the atmosphere in copper metallurgy are metallurgical furnaces, refining processes technologies. Connected with waste utilizing and transport devices.

The purpose of this investigation was estimation of the level of contamination with selected chemical elements possessing toxic properties (Pb, Cu, Cd, Zn) of bee's honey originating from copper industry and rural-forest region.

MATERIAL AND METHODS

The investigation was conducted in 2005 in two regions differing in the degree of anthroporession on natural environment:

1. copper industry – Legnica and Głogów copper district – experimental region
2. agricultural and forest – the Kłodzko basin – control region.

The material for examination were the samples of multiflower honey collected, after centrifuging, as average samples from particular apiaries. Sample collection took place in July and August 2005. Total number of honey samples collected from those regions was 24. After sample unification out of each sample there was prepared a weighed portion of 1000 mg ($\pm$0.10 mg). Those honey portions underwent chemical decomposition with concentrated nitric acid, spectrally pure (Merck company, Germany), 10 ml per each sample, and then they were mineralized under high pressure in microwave oven, Mars 5 by CEM firm, where sample decomposition was conducted in closed non-stick containers. Quantitative analysis of multiflower honey regarding the content of trace elements such as: copper, cadmium, lead and zinc was done using ICP plasma spectrometer operated by P-3202 computer cooperating with analytical device Philips Scientific model PU-7000 and CETAC-5000 AT supersonic nebuliser. Calibration curves for particular chemical elements were drawn using the patterns ICP class, featuring special purity. The results obtained were statistically worked out using computer program Statgraphics version 5.1 – there were calculated average contents of metals in particular regions of investigation, standard deviations and correlations between content levels of particular elements. Difference significance in chemical elements content between the regions under examination were estimated according to Duncan multiple spread test.

RESULTS AND DISCUSSION

The results of our examination proved that the level of heavy metals content in honey samples coming from copper industry region was lower than permissible norm (tab. 1). Copper is a dominating chemical element in Legnica and Głogów copper district, yet the examinations proved that its average concentration was the lowest just in the honey from the mentioned region and it amounted 0.484 $mg \cdot kg^{-1}$ d.m. (tab. 1). It was higher, however, in control samples – 1,353 $mg \cdot kg^{-1}$ d.m. Low copper content in bee's honey also confirm Lipińska and Zalewski [1989]: from 0,69 to 2,33 $mg \cdot kg^{-1}$. Buliński et al. [1995] proved that mean copper content ranging from 0,14 $mg \cdot kg^{-1}$. Comparable data were reported by Szkoda and Żmudzki [2002], as well as by Przybyłowski et al. [2003]. Perużyński [2003] also observed significantly low copper content – 0,320 $mg \cdot kg^{-1}$ d.m. in the honey collected from the area situated about 30 km away from chemical plant in Police. Dobrzański et al. [1994] detected low level of copper concentration in the honey from copper industrial region – 0.194 $mg \cdot kg^{-1}$, while Roman [1997] recorded maximum copper concentration in the same region amounting 15.820 $mg \cdot kg^{-1}$ d.m.

Elevated cadmium content can occur in the honey from typical agricultural regions, where high consumption of mineral fertilizers and plant pesticides often takes place. That fact can be average 0.373 mg·kg^{-1} d.m. (tab. 1). In copper industrial region mean cadmium concentration was significantly low – 0.041 mg·kg^{-1} d.m. In the available literature similar low values were obtained by Migula et al. [1992] – 0.043 mg·kg^{-1} ore-bearing area, Perużyński [2003] and Stein and Umland [1986] – 0.014–0.052 mg·kg^{-1}. Low cadmium content was also recorded by Roman [1997 and 2000] in the samples from copper industry region. Higher level of cadmium accumulation was reported by Przybyłowski [2003] in nectar honey – 0.113 mg·kg^{-1}, while in honeydew ones it amounted 0.346 mg·kg^{-1}.

Zinc belongs to microelements, which are indispensable for appropriate course of live processes, as it is a component or activator of numerous enzymes. Yet its excessive amounts can be harmful to people and animals, or even toxic [Kabata-Pendias and Pendias 1999]. Our investigation proved that in honey samples from copper industry region zinc content ranged average 3.343 mg·kg^{-1} d.m. and maximum valve – 11.891 mg·kg^{-1} d.m. (tab. 1). Lower mean concentration value of this metal was recorded in control region honey – 3.179 mg·kg^{-1} d.m. In the available literature there are reported considerable discrepancies regarding zinc level in honey. Perużyński [2003] proved average 1.242 mg·kg^{-1} d.m., while Buliński et al. [1995] – 9.90 mg·kg^{-1} d.m. (the highest value – 30.0 mg·kg^{-1} d.m.). Similarly, Lipińska and Zalewski [1989] report of zinc content amounting 19.70 mg·kg^{-1}, while Gajewska et al. [1984] – even 49.1 mg·kg^{-1}. Roman [1997] detected average 19.53 mg·kg^{-1} d.m. in the honey from copper industrial region and maximum value ranged 177.91 mg·kg^{-1} d.m.

Our investigation proved that the highest lead content occurred in the honey from control region, where mean value was 0.147 mg·kg^{-1} d.m. and maximum – 0.980 mg·kg^{-1} d.m. (tab. 1). In Honey samples from copper industry region there was determined very low lead concentration, ranging 0.062 mg·kg^{-1} d.m. Comparable lead values in bee's honey were reported by Szkoda and Żmudzki [2002]: 0.001 to 0.251 mg·kg^{-1}. Similar results were obtained by Buliński et al. [1995] – 0.115 mg·kg^{-1} according to Lipińska and Zalewski [1989] lead content in nectar honey ranged from 0.057 to 0.370 mg·kg^{-1}. Madras-Majewska and Jasiński [2003] recorded lead vale from 0.005 to 0.1469 mg·kg^{-1}. Perużyński [2002] also obtained low lead amounts in bee's honey originating from West Pomeranian region – 0.216 mg·kg^{-1} d.m. Accorti et al. [1990] recorded mean lead concentration 0.210 mg·kg^{-1} and Bogdanov et al. [1986] 0.09 mg·kg^{-1} in honey samples coming from Italy. Increased values of lead in copper industry region were detected by Dobrzański et al. [1994] average values ranged from 0.369 to 1.025 mg·kg^{-1} d.m., I.E. they were higher than those obtained in our own investigation regarding that region. Also in the work by Roman [1997] lead accumulation level in the honey from Legnica and Głogów copper district proved to be higher and mean values ranged from 0.465 to 1.097 mg·kg^{-1} d.m.

On the basis of the results obtained there were proved statistically significant differences (at $P_{0,05}$) in copper and cadmium concentration between the regions examined (tab. 1). In honey samples from both regions there were recorded significant correlations (at $P_{0,01}$) between the level of cadmium and lead concentration. Similarly significant correlations (at $P_{0,05}$) were proved between the level of copper and cadmium concentration in the honey from Legnica and Głogów region and between copper and zinc concentration in control region honey (tab. 2). The results of our investigation proved that in experimental region mean contents of the examined chemical elements were lower (except for zinc) than those obtained from control region (tab. 1). It can be concluded that the mentioned situation resulted from considerable improvement of the state of natural environment in Legnica and Głogów region in the recent years, as well as from high

ability of purification of honey raw material from trace metals in the course of its processing into honey performed by bees themselves [Roman and Demeńczuk 2003].

CONCLUSIONS

1. In the examined honey samples different concentration there was recorded of selected chemical elements bearing toxic properties (Cu, Pb, Cd and Zn).
2. Average content of particular metals in the honey coming from copper industry region did not exceed permissible concentration set by The Polish Norm.
3. In agricultural and forest region dominating element in bee honey occurred to be cadmium – its mean concentration was three times higher than the norms in force.
4. Regarding bee honey as a bioindicator of environmental pollution with heavy metals it should be stated that the region under copper industry effect can be regarded as not endangered ecologically.

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Table 1. Concentration level of the examined heavy metals in multiflower honey (in $mg \cdot kg^{-1}$ s.m.)

Sample number	Cu		Cd		Pb		Zn	
	LGOM	Control	LGOM	Control	LGOM	Control	LGOM	Control
1	1,375	1,152	0,025	0,516	0,020	0,010	4,678	2,260
2	0,131	1,067	0,070	0,044	0,048	0,032	1,557	1,679
3	0,115	1,347	0,017	0,087	0,035	0,023	1,247	2,385
4	0,126	2,203	0,064	1,978	0,105	0,980	1,770	3,269
5	0,323	0,116	0,093	0,055	0,081	0,080	2,213	1,619
6	0,116	0,100	0,061	0,099	0,076	0,115	1,254	0,823
7	1,565	0,122	0,045	0,080	0,067	0,054	1,618	2,851
8	1,256	0,693	0,023	0,122	0,042	0,089	2,478	6,613
9	0,324	4,651	0,064	0,087	0,039	0,077	8,396	7,762
10	0,134	2,115	0,080	0,099	0,045	0,042	11,891	4,111
11	0,222	1,448	0,102	1,218	0,105	0,203	1,356	2,331
12	0,124	1,224	0,070	0,096	0,076	0,061	1,652	2,447
Minimum	0,115	0,100	0,017	0,044	0,020	0,010	1,247	0,823
Maximum	1,565	4,651	0,102	1,978	0,105	0,980	11,891	7,762
Average	**0,484**^{A}	**1,353**^{B}	**0,059**^{A}	**0,373**^{B}	**0,062**	**0,147**	**3,343**	**3,179**
SD	0,560	1,203	0,027	0,582	0,027	0,256	3,393	1,974
NDS [1)]	12,00		0,12		0,50		18,00	

[1)] The highest permissible concentration (The Polish Norm, 1988)
A, B – statistically significant differences at $P_{0,01}$

Table 2. The values of correlation coefficient between the examined elements (n=12)

Chemical elements	Region under investigation	
	Copper industry region	Agricultural and forest region
Cu → Cd	–0,556 *	0,198
Cu → Pb	–0,367	0,204
Cu → Zn	–0,053	0,686 **
Cd → Pb	0,667 *	0,887 **
Cd → Zn	0,126	–0,070
Pb → Zn	–0,440	0,013

* – significant coefficient of correlation at $P_{0,05}$ level
** – highly significant coefficient of correlation at $P_{0,01}$ level.

AN EPIDEMIC OF SALMONELLOSIS CAUSED BY SILAGE CONTAINING SALMONELLA AT A DAIRY FARM

Ruoho, O.

The Association for Animal Disease Prevention ETT ry, Seinäjoki, Finland

SUMMARY

The aim of the project was to eradicate salmonellosis in a loose-housing dairy herd. The ground principle of the eradication program is to assure good hygienic quality of feed and drinking water and to cut the infection chain from faeces to feed. During the eradication procedure it came up that the farm had been chronically infected with salmonella at least a year. Contaminated slurry had been spread on a field for silage production and the silage had been contaminated. When contaminated silage was fed to animals, it caused a new outbreak of salmonellosis, although special hygienic measures were kept on during the eradication procedure.

Keywords: contamination, dairy farm, enteric disease, eradication procedure, faecal samples, hygienic measures, infection chain, loose-housing system, silage, swab samples

INTRODUCTION

An epidemic of salmonellosis caused by *S. muenchen* was found in February 2006 at a dairy farm in Southern Finland. The infection was detected, when several calves had an enteric disease and samples from some dead calves were examined at a laboratory. The farm has a warm loose-housing system and about 120 dairy cows and 250 young animals. In Finland all farms detected to be infected with salmonella are put under restrictions by the authorities. The eradication program for salmonellosis was started at the farm immediately. The farm has insurance for the costs of salmonella eradication for the first half year since start of the restrictions.

ERADICATION PROCEDURE

The ground principle of the eradication procedure is to assure good hygienic quality of feed and drinking water and to cut the infection chain from faeces to feed. To investigate the situation all animals at the farm were examined at the beginning on March 2006 for salmonellosis by individual or pooled faecal samples. Individual samples were taken from the cows in the loose-housing system and pooled samples from the pens for young animals. In addition, several swab samples were taken from the production environment; entrance of the cow house, dairy room, corridors, feeding tables, drinking vessels, feeding equipment and feed stores. The faecal and swab samples were examined at the laboratory according to the NMKL 71/1999- method.

The animals at the farm are fed with TMR (total mixed ration) –system and the feed is delivered by a TMR-car pulled by a tractor. All feed components used for TMR and the TMR-feed itself were examined according to the ISO6579,2002-method for salmonella. The main

component of the TMR-feed is pre-dried silage harvested from the own fields of the farm. Swab samples were taken from the TMR-car and the tractor pulling it.

At the first sampling about 15% of the animals were positive for salmonella. Some swab samples taken from feeding tables and drinking vessels were also positive. All feed samples were negative for salmonella. The hygienic procedures were concentrated to the critical points measured by the results of the swab samples taken from the production environment. Feeding tables and drinking vessels were disinfected twice a day. The wheels of the TMR-car and tractor were disinfected every time before driving to the feeding table. It was not possible to isolate the positive for salmonella animals, neither considered necessary to remove those from the herd at this stage.

The progress of the eradication procedure was monitored by faecal samples taken at the interval of two to four weeks. A farm is considered to be free of salmonellosis, when two successive faecal samples from the whole herd are negative. The next faecal samples were taken at the beginning of April and only one young beef animal was found positive for salmonella. The animal was slaughtered at the farm and sent for destruction. The slaughterhouses in Finland do usually not take animals from farms that have restrictions because of salmonellosis. Milk is usually delivered to dairy, if the quality is good and the farm has no problems with milking hygiene.

Of the faecal samples taken at the end of April 2006, however, 30% were again positive for salmonella. For this reason new samples from the production environment and the feed were taken. Two swab samples taken from the bottom of a silage store opened and finished after the first sampling at March were positive for salmonella. The storage was already empty, so it was not possible to get a sample from the silage. Of the swab samples taken from the production environment itself only one swab sample taken from the feeding table was found positive. The hygienic measurements were further kept on.

When feeding with the contaminated silage was stopped the animals got rid of salmonellosis in one month. Faecal samples taken at the end of May and at the end of June were negative for salmonella and the restrictions were released. The silage store was washed and disinfected properly before the next harvest season.

The farm had four storages for liquid manure near the cowhouse and one storage at a distance of ten kilometres in the middle of the fields. The farmer usually transports the liquid manure to the storage located at the fields during the winter-time. All storages were examined for salmonella and found positive. The liquid manure was disinfected by mixing 30 kg lime per 1000 litres manure so that the pH of the manure was over 11. After that the slurry was ploughed into the field. The field was not used for silage production during the first year.

CONCLUSIONS

In the salmonella infected storage the silage harvested in summer 2005 was produced from a field, where in the same spring liquid manure had been spread. Hence, we can conclude that the farm seems to have been infected by salmonella already during the winter 2005. Salmonella had then been excreted into the liquid manure spread on the field mentioned before. The acidity in predried silage is not low enough to destroy salmonella in the silage. It always is a threat to feed hygiene to spread liquid manure on grassland for silage production especially if the status of salmonella at the farm is unknown.

Table 1. Cattle Farms under restrictions for salmonellosis in Finland; year 2005–2006

Serotype	**Number of cases (farms)**
– *S. infantis*	1
– *S. Konstanz*	1
– *S. muenchen*	1
– *S. typhimurium FT 1*	4
– *S. typhimurium FT 2*	1
– *S. typhimurium FT 9 var*	1
– *S. typhimurium FT 41*	3
– *S. typhimurium FT NST*	4
	Total:16

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SEROLOGICAL DIFFERENTIATION BETWEEN ACUTE AND CHRONIC STAGES IN EXPERIMENTAL *TOXOPLASMA* INFECTION OF NORMAL AND IMMUNOSUPRESSED RATS (*Rattus norvegicus*)

Silva, R.C.[1,2], Da Silva, A.V.[2], and Langoni, H.[2]

[1] Master scholarship holder, FAPESP 03/08063-0, Brazil; [2] Zoonosis Researches Nucleus (NUPEZO). Faculty of Veterinary Medicine and Animal Husbandry (FMVZ), São Paulo State University (UNESP). Distrito de Rubião Júnior, s/n, Botucatu, SP. 18618000; Tel.: + 55 14 38116270. Fax: + 55 14 38116075. E-mail: silva_rcd@fmvz.unesp.br

SUMMARY

The immune response of toxoplasmosis can give good information to an accurate vigilance of food products. This study analyzed anti-*Toxoplasma gondii* antibodies kinetics, with or without immunosuppression. Three groups of four rats, G1 and G2 infected with bradyzoites, G2 immunosupressed, and G3 control, were evaluated weekly for modified agglutination test (MAT), with methanol (AC) or formalin (HS), and immunofluorescent antibody test (IFAT). IFAT did not differentiate acute and chronic stages. MAT-AC detected antibodies only from acute stage, while MAT-HS from both stages. Thus, the differentiation of the stages is possible from sequenced sera samples.

Keywords: *Toxoplasma gondii*, *Rattus norvegicus*, serology, experimental infection

INTRODUCTION

Toxoplasma gondii is an obligate parasite protozoan, worldwide distributed, that infects warm-blooded hosts, including human beings. It has a great importance to immunocompromised patients being an opportunistic parasite, causing serious sequels like toxoplasmic encephalitis, and to pregnants, causing fatal damages to fetus (Dubey & Beattie, 1988; Smith, 1997). The hosts can be infected for the consumption of raw and undercooked meat of a chronic infected animal containing cyst with bradyzoites, food or water contaminated with sporulated oocysts eliminated for infected cats in its faeces, or for transplacental transmission of tachyzoites (Tenter et al., 2000).

In immunocompromised patients, the reacutization of chronic infection occurs due the low cellular and humoral responses, releasing bradyzoites from tissue cysts that convert in tachyzoites, dissemining to all host tissues, i.e., brain, lung, heart, liver, spleen (Carruthers, 2002; Luft & Hafner, 1990).

Tachyzoites and bradyzoites of *T.gondii* have in them surfaces specific antigens. Modified agglutination test (MAT) allows detecting specific antibodies from these antigens of the acute and chronic stages, of any species, only dependent of antigen-antibody interaction. While this, methanol (MAT-AC) exposes specific antigens from acute stage (tachyzoites, 24KDa), not presenting crossed-reaction with bradyzoites, while formalin (MAT-HS) exposes both antigen

from acute and chronic stages, i.e., bradyzoites 35Kda antigen (Dubey & Beattie, 1988; Thulliez et al., 1986).

This study aimed to analyze experimentally the dynamic of antibodies and the applicability of serological tests for the diagnosis of toxoplasmosis, comparing the evolution of antibodies in infected rats, immunosupressed or not with corticoids, and measuring specific antibodies from each stage.

MATERIAL AND METHODS

All experimental study was realized in the laboratories of the Zoonosis Researches Nucleus, FMVZ, UNESP, Campus of Botucatu, SP.

Three experimental groups were used in this study. G1 and G2 groups, four Wistar rats (*Rattus norvegicus*) each groups, infected with 10^4 bradyzoites of BTU 10 strain, genotype I, p.o., being that G2 was immunosupressed with corticoid (Dexamethazone, p.o. + Hydrocortisone succinate, s.c.) since 155th day after infection, according to the protocol described for Djurkovic-Djakovic & Milenkovic (2001), adapted by interspecific allometric extrapolation protocol (Pachaly & Brito, 2001); G3, four Wistar rats inoculated with saline solution, as control.

The blood samples were collected weekly, during 90 days (G1 and G3), and 180 days (G2), by the punction of the orbital sinus. The samples were centrifuged at 1600g per 10 minutes, and the sera samples were frozen until the moment of the test.

The serological tests used were the modified agglutination test (MAT) and immunofluorescent antibody test (IFAT).

MAT-HS (Desmonts & Remington, 1980) using formalin, and MAT-AC (Silva, 2006), using methanol to fix the antigens, were realized to measure titres of antibodies from both stages, and to analyze the reacutization of the infection. A clear-cut button-shaped deposit of parasite suspension at the bottom of the well was considered negative, and a complete carpet of agglutinated organisms was positive (Figure 1).

IFAT (Camargo, 1974) was realized using FITC anti-rat-IgG and FITC anti-rat-IgM (Bethyl laboratories ®), according to the instruction of the manufactory, in a Zeiss SH250 fluorescence microscope, 40x. An intense fluorescence in all membrane of tachyzoites in 50% or more, per field, was considered positive to this dilution, until the final dilution of the test.

All sera samples were evaluated to MATs and IFAT. The titres of antibodies were converted in log (10*titre), and the area under the curve of the titres (AUC) obtained (Jungersen et al., 2002). AUC of different serological tests were compared by Student t Test, to compare two tests, or by One Way Analysis of Variance, with comparison of averages by Tukey test. The comparison of the AUC of the intervals of weeks for each one test was realized for the Analysis of Variance for Paired Groups, with comparison by Tukey test.

RESULTS AND DISCUSSION

To both groups, G1 and G2, IgM titres were identified already since the first days, with higher levels than IgG until 14 days of infection, where IgG starts the production and display equal or higher levels than IgM between the 3rd and 4th weeks. Naot & Remington (1980) verified the same, but comparing ELISA-IgM and IFAT-IgM, where basal level of IgG is produced, when a peak of IgM is present.

Between the 2nd and 3rd weeks, IgM and IgG display same levels. This occurs because the immune response of the host was researching of a better form to combat the infection, and with IgM, in the first days is essential due the rapid production but with low specificity. With the progressive maturation of the infection, a specific immune response is produced, mainly with IgG.

After the 3rd and progressive weeks, a same response of antibodies was observed in both groups. IgM and IgG maintained the same level with a discrete and progressive decline of IgM, which is observed with IgG too, but with minor intensity. In G2, important data could be observed in relation to the differentiation of antibodies. IgM and IgG could not be differentiated, in rats, after the 3rd week, along of 23 weeks studied.

To IFAT is important to consider that, in rats, IgM can give an important indication of acute infection. But we should to consider too that the immune response in toxoplasmosis, is very specify, and *T.gondii* epitopes are very immunogenic. So that, the immune response is very intense, maintaining higher titres of antibodies for a long time. Thus, higher titres of IgM and IgG can be observed for many weeks, as observed in this study. With this, and observing the Graphic 1, the differentiation between acute and chronic stages, based in IgM and IgG, in rats, is very difficult after the first weeks.

Evaluating MATs at the final of the 2nd week, IgG starts to be detected, and until the same week only IgG from acute stage (IgG-AC) was detectable. Between the 2nd and 3rd weeks, IgG-AC presented lower levels than total IgG detectable, being this final IgG the IgG-AC added of those from chronic stages, which was in lower quantity in the studied period, agreeing with Thulliez et al. (1986).

Along the 13th weeks, the differentiation increased characterizing that the maturation of the immune response starts early, in minor intensity, and is occurring, advancing for a long time. This fact can be better observed in G2, along of 23 weeks. However, MAT allows the differentiation between acute and chronic stages, as studied for Dannemann et al. (1990).

A peak of antibodies was identified between the 3rd and 4th weeks. After this, IgG levels reduced gradually, most emphasized in those from acute stage, which demonstrate the maturation gradually of the immune response.

Both MAT-AC and MAT-HS presented significant difference ($P<0.05$) between both groups, G1 and G2. MAT-HS differed significantly to IFAT-IgG and to IFAT-IgM ($P<0.05$). In contrast, both IFAT tests not differed significantly each other ($P>0.05$), as well as MAT-AC and IFAT-IgG to G1, which occurred to G2 ($P<0.05$). All animals of G3 were negative serologically.

After the immunosupression, in G2, at 23rd week was observed an increase of IgG, to IFAT-IgG as well as MATs, but most emphasized in MAT-HS. Curiously, after the introduction of immunosupression, in IFAT-IgG was observed a rapid decline, followed for an increase of antibodies. This fact could be explained for rapid sensitization of the immune system, followed for a recuperation and elevation in antibodies levels at the immune response. Djurkovi-Djakovic & Milenkovic (2001) verified that the prolonged use of corticoid may cause considerable toxicity, with progressive loss of weight and death.

In the Table 1, only IFAT-IgG differentiate significantly the last period, 22nd to 26th week, to the 4th period, 17th to 21st week, which deserve other studies to characterize this reacutization, because only this test had detected, statistically, the immunosupression serologically.

To differentiate the stages of *T.gondii* infection and to contribute effectively to public health (Desmonts & Remington, 1980; Marca et al., 1996), a period upper than six months should be, with paired sera samples considered, allowing the characterizing of the behavior of the antibodies anti-*T.gondii* along the infection.

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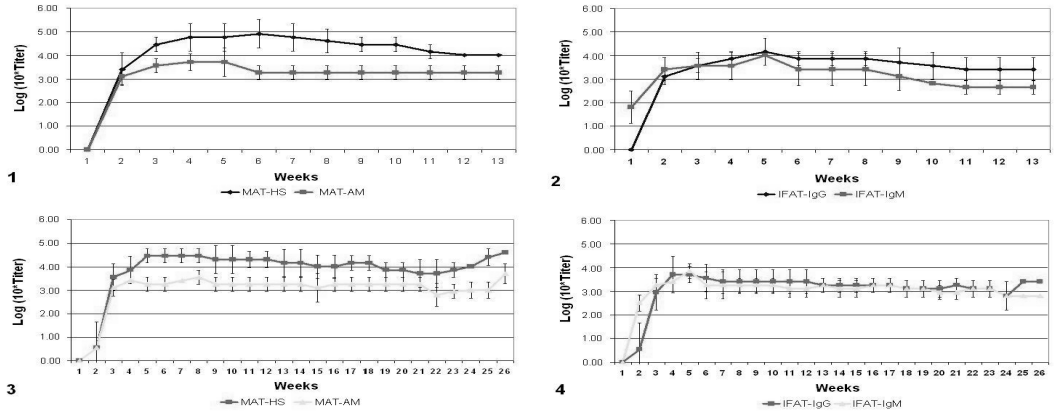


Graphic 1. Week variation of the concentration of anti – *T.gondii* antibodies in immunosupressed or not experimentally infected rats. Botucatu, 2006

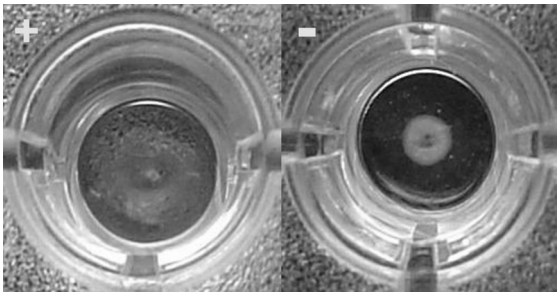


Figure 1. Positive (left) and negative (right) microscopic evaluation for MAT. Botucatu, 2006

Table 1. Comparison among the means of curves of the concentration of antibodies, in different intervals, in animals submitted to immunosupression, according to the serological test used. Botucatu. 2006

Test	Periods / Mean ± Standard deviation of the area of the curve					F Statistics	P Value
	1st a 6th week	7th a 11th week	12th a 16th week	17th a 21st week	22nd a 26th week		
MAT-HS	$2.44^{A} \pm 0.34$	$3.49^{B} \pm 0.35$	$3.30^{B} \pm 0.39$	$3.16^{AB} \pm 0.23$	$2.88^{AB} \pm 0.59$	1.7140	0.0146
MAT-AC	$1.99^{A} \pm 0.26$	$2.68^{B} \pm 0.18$	$2.58^{AB} \pm 0.30$	$2.61^{AB} \pm 0.24$	$2.15^{AB} \pm 0.51$	1.4160	0.0156
IFAT-IgG	$2.12^{A} \pm 0.45$	$2.73^{AB} \pm 0.39$	$2.62^{AB} \pm 0.25$	$3.15^{B} \pm 0.28$	$2.22^{A} \pm 0.55$	4.1360	0.0038
IFAT-IgM	$2.44^{A} \pm 0.13$	$2.59^{A} \pm 0.24$	$2.53^{A} \pm 0.24$	$2.46^{A} \pm 0.21$	$2.12^{A} \pm 0.31$	1.7830	0.0719

Statistical results: values of P less than 0.05 indicate significant difference among the curves of concentration of antibodies in the studied periods, for Analysis of Variance to dependent samples – value of averages followed by different words indicate significant differences among the periods, to one test of detection of antibodies, considering a significance level of 5%.

THE INCIDENCE OF HEAVY METALS IN THE ONE POND AREA AROUND OF BUCHAREST AND IN FISHES THAT POPULATED THESE WATERS

Simion, V.-E.[1], Mitrănescu, E.[2], Pădure, A.[1] and Tăpăloagă, D.[2]

[1] The Veterinary Medicine Faculty, Spiru Haret University, Jandarmeriei Street, no.2, Bucharest, Romania, e-mail: liviusimion@b.astral.ro; [2] The Veterinary Medicine Faculty, USAMV University, Splaiul Independenței Street, no.105, Bucharest, Romania, email: mitranescuelena@gmail.com

SUMMARY

The study aimed at determining the concentration of two heavy metals, in the water of a pool from Ilfov County, by the atomic absorption spectroscopy.

The Pb content in the assays of water varied in the limits between 3.43 and 6.21 μg/l, and the content in Cd in the assays of water varied in the limits between 0.02 and 0.25 μg/l. The analysis of muscular tissue taken from the fish living in this pool relieved content in Pb which varied between 0.04 and 0.81 ppm and content in Cd between 0.05 ppm and absent.

The diversity of values registered due to some factors does not show a correlation between the concentration of metals in water and in the muscular tissue of fish but the cumulative effect of these represents a risk for the human population.

Keywords: lead, cadmium, fishes, pond area

INTRODUCTION

Water is an important natural resource for men, both directly and indirectly, by means of the fish resources. In comparison with the other species of animals, fish use as sources of nutritive substances, both food and water from the environment where they live. Both nutritive substances and pollutant substances enter the fish body through the gills and through the hundreds of capillaries existing at this level. The sources of water can be constantly polluted with a series of metals (as well as with other pollutants) which can be sources of intoxications for men, depending on the dose of metal that exists in water. Metals have both benefits for the human body, when they act as mineral substances and also toxic effects when they reach a certain concentration. Cadmium (Cd) is considered to be a toxic metal for fish, its absorption at the level of the gastro-intestinal tract increases the incidence of the hepatic necrosis and mortality (NRC, 1993). Lead (Pb) affects pituitary function, gonadosomatic index, oocyte growth, neurological disorders, and scoliosis [3].

Carp (*Cyprinus carpio*) is an omnivorous species and in its natural environment it feeds on: shrimps, mud worms, the larva of some insects but also the seeds of the plants living under the water, plants ramie or different parts of plants in different stages of putrefaction (Laszlo).

The correlation between heavy metals and the content of organic sediments in heavy metals, the source of food at this species, is well known (*Van-Hattum* and col., 1993). Fish is known for

the bioaccumulation of the heavy metals in its body, thus it is an important biomonitor of their presence in water.

In the rural areas, fish is an important source of food for the human population and its procurement is not always controlled, therefore there is more than often a risk for those people to consume contaminated fish.

MATERIAL AND METHODS

The study focused on the analysis of the concentration of two heavy metals – Lead (Pb) and Cadmium (Cd) in 10 assays of water taken from the mere of Branita village, Ilfov County, close to Bucharest. The assays were taken from different depths, both from the surface of the lake and from the bottom of water. The analysis of the concentration in Cd and Pb from the water was made by means of the atomic absorption spectroscopy, adjusting the wave length at 228.8 nm for Cd and 283.3 nm for Pb.

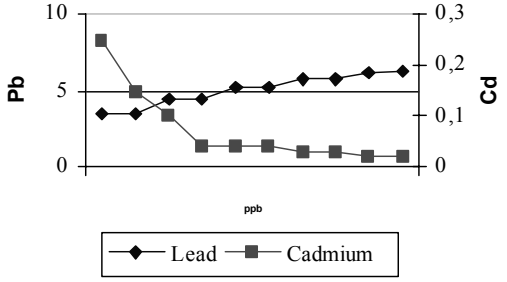
At the same time, 10 exemplars of fish from the species of *Cyprinus carpio* were fished in order to investigate the concentration in Pb and Cd. After the fish assays were processed, the concentration in the two metals was performed by means of the atomic absorption spectroscopy, adjusting the wave length at 328.1 nm for Cd and 217 nm for Pb.

RESULTS AND DISCUSSION

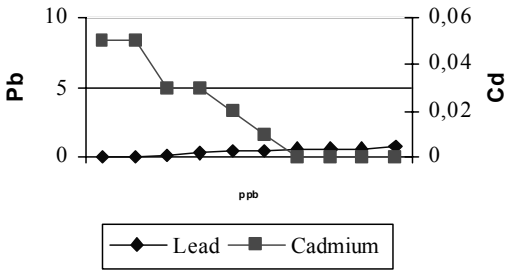
After the analysis of the 10 assays of water which were taken from a mere from Ilfov, the following results were registered (Graphic no. 1): the content in Pb of the assays of water varied between 3.43 and 6.21 µg/l, the maximum allowed limits were between 1–5 µg/l; the content in Cd in the assays of water which were analyzed varied in the limits of 0.02–0.25 µg/l, the maximum allowed limits were between 0.1–0.5 µg/l (Chart no. 1).

Table 1. The content in lead and cadmium in the analyzed water assays

No. assays	Pb µg/l	Level of pollution	Quality class	Cd µg/l	Level of pollution	Quality class
1	3.43	+	IV	0.02	–	I
2	3.50	+	IV	0.02	–	I
3	4.41	+	IV	0.03	–	I
4	4.43	+	IV	0.03	–	I
5	5.20	+	V	0.04	–	I
6	5.25	+	V	0.04	–	I
7	5.78	++	V	0.04	–	I
8	5.80	++	V	0.10	+	II
9	6.10	+++	V	0.15	+	II
10	6.21	+++	V	0.25	++	III



Graphic 1. The Pb and Cd content in the assays of water



Graphic 2. The Pb and Cd in the assays of muscular tissue

According to the Directive 76/464/CEE regarding the pollution caused by certain dangerous substances from the aquatic environment, the classification of the water quality is made in quality classes, classes II – V for Pb representing concentrations between 1 and > 5 μg/l and for Cd, concentrations between 0,1 and > 0,5 μg/l.

After the analysis of the 10 assays of muscular tissue coming from the sweet water fish (C*yprinus carpio),* the following results were registered (Graphic no. 3): the content in Pb varied between 0.04–0.81 ppm, the allowed limits were between 0.2–0.4 ppm; the content in Cd of the fish assays was between 0.05 ppm and absent, the maximum allowed limits are 0.05 ppm (Chart no. 2).

Table 2. The content in lead and cadmium in the assays of muscular tissue coming from the sweet water fish (*Cyprinus carpio)*

No. assays	Pb (ppm)	Level of pollution	Cd (ppm)	Level of pollution
1	0,04	–	absent	–
2	0,05	–	absent	–
3	0,10	–	absent	–
4	0,25	+	absent	–
5	0,50	++	0,01	–
6	0,51	++	0,02	–
7	0,53	++	0,03	–
8	0,60	+++	0,03	–
9	0,62	+++	0.05	+
10	0,81	++++	0,05	+

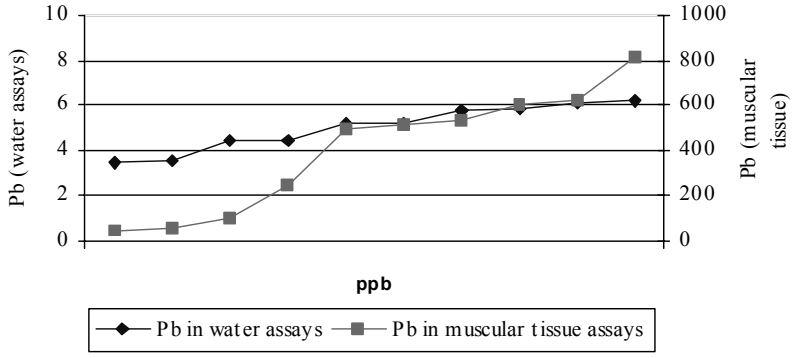
Lead is one of the heavy metals, regarding which there are many discussions and according to the Decision of the International Organization for Health Protection, Pb is given to the first indices of evaluation of the environment pollution.

Pb is one of the four metals which have the most destructive effect on human health. It can enter the human body by means of food (65%), water 20% and air (15%). The contamination of man with Pb is most often made by ingestion after the consumption of fish. The minimum allowed concentrations of Pb in the alimentary products vary between 0.005 mg/kg in the diary products and 1.0 mg/kg in fish. Fish meat may contain Pb especially if the environment in which those fish live, is extremely contaminated. The accumulation is more frequent at the rapacious

fish, especially due to the cumulative effect and to the contamination "in pyramid" (they consume other potentially contaminated fish). Lead is deposited especially in liver and muscles, the older the fish are, and the more important the deposits are.

The toxicity of Pb through fish consumption depends on the chemical form in which Pb is, thus when it is in its inorganic form, Pb from fish is absorbed by man in proportion of 10%, while in its organic form of tetrametil of Pb, it will be absorbed in proportion of 100% (*Allan Johnson*, 1996).

In Graphic no. 3 there is a comparative presentation of the Pb concentrations in water and in the muscular tissue of the fish which were taken from that water.

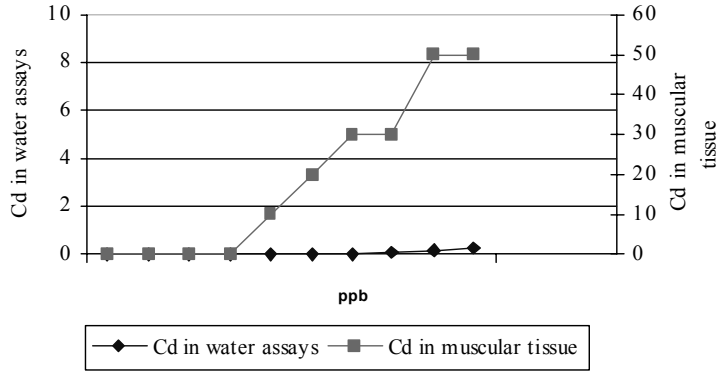


Graphic 3. The content in Pb in the assays of water and muscular tissue

Cadmium represents a chemical composite without an essential biological function, since it is known the fact that cadmium can be considered one of the most toxic heavy metals. Cadmium is fast stored in the fish body, but its metabolism and elimination happen slowly. Cadmium enters the fish body through the gills because it has a negative load, fact which induces a great affinity for the ions of the metals that have a positive load.

The concentration of Cd in fish is of about 20μg/kg wet weight, the exposure of fish to the artificial intoxication with Cd, shows that the main effects of the intoxication are the lack of movement at fish, while the response to light and sounds is obviously weaker. The election organ for the deposits made by cadmium in fish is the kidney (0.402 μg/g), followed by the digestive tract, skin, etc [1]. The accumulation period for the cadmium is of 4–7 days, after that its elimination starts. In the case in which the concentrations in fish are much higher than the admitted limits, there is a second period of redistribution in the organs of the cadmium ions (14 – 30 days) and then a new period of elimination starts [2].

In the Graphic no.4 there is a comparative presentation of the Cd concentrations in water and in the muscular tissue of the fish taken from that water.



Graphic 4. The content in Cd in water assays and muscular tissue

Since Cd is accumulated in the organs and it has quite a long period of semi-elimination (10 – 30 years), the use of insignificant quantities of fish containing Cd during a long period of time, can lead to some intoxication forms. The regulating standards limit the use of fish with cadmium content Cd > 0.5 mg/kg dry weight. This also draws the attention that liver and other organs from fish are not good for consumption.

The chronic toxicity tests performed on fish, showed that 1.7 μg/kg represents the lowest value at which the chronic intoxication of fish occurs. According to standard regarding certain contaminants from the food of animal and non animal origin, the maximum allowed level in fish fillet is for Pb of 0.2 mg/kg and for Cd of 0.05 mg/kg.

CONCLUSIONS

Both cadmium and lead, due to their capacity of accumulation in the environment and in the organisms living in that environment and due to the harmful effects that they have upon the health of the aquatic animals are considered to be two of the heavy metals with a high degree of toxicity.

The Pb content in the assays of water taken from different areas varied in the limits between 3.43 and 6.21 mg/dm^3 and the content in Cd in the same water assays varied in the limits between 0.02 and 0.25 mg/dm^3.

The analysis of 10 assays of muscular tissue taken from the fish living in this pool relieved content in Pb which varied between 0.04 and 0.81 ppm and content in Cd between 0.05 ppm and absent.

The results that were obtained confirm for water an average pollution with Pb, 6 of the 10 assays had over 5 μg/l (class V of quality) and almost the lack of pollution for Cd, 7 from the 10 assays had under 0.1 μg/l (class II of quality).

The concentration of metals in fish was higher for Pb, only 3 assays of 10 had under the limit of 0.2 ppm and even more reduced for Cd, 8 assays of 10 had under 0.05 ppm.

Due to its anatomic and physiological particularities, fish is one of the species with a high contamination risk with heavy metals and at the same time, it is a potential toxin factor for the human population.

The diversity of value registered due to some factors (the fish exemplars, the pool area, the aquatic flora) does not show a correlation between the concentration of metals in water and in the

muscular tissue of fish but the cumulative effect of these represents a risk for the human population.

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F –
EMISSIONS AND WASTES IN ANIMAL HUSBANDRY – RISKS FOR ANIMAL AND HUMAN HEALTH

ORAL PRESENTATIONS

STRATEGIES FOR HYGIENIC SAFE RECYCLING OF ORGANIC WASTES AND RESIDUALS TO AGRICULTURE

Böhm, R.

Universität Hohenheim, Institut für Umwelt und Tierhygiene sowie Tiermedizin mit Tierklinik D – 70993 Stuttgart, Germany

SUMMARY AND RECOMMENDATIONS

Certain health-risks for man, animals and plants due to pathogens and other microorganisms with undesired properties are connected with the utilization of wastewater, sewage sludge, organic wastes and residuals as well as animal by-products. Those risks can be minimized by adequate measures in collection, transport, storage and treatment, best in the framework of a HACCP-concept. The first step in the proposed HACCP concept is the analysis of the epidemiological situation concerning diseases of man, livestock and plants in the area the raw materials that are originating from (hazard analysis). This has to be followed by validation of the intended treatment process with test organisms covering the resistance of the "key-pathogens" identified in the hazard analysis accompanied by measuring the technical data in the process that are relevant for the inactivation of the pathogens and test organisms at representative places in the equipment (critical control points). Keeping of the data set in the validation process is verified by continuously measuring the relevant parameters at the critical control points and by filing the data for at least two years. Finally, the treated product has to be examined for presence and absence of raw material dependent relevant indicators also identified by taking the results of the validation procedure into account. If the necessary degree of hygienic safety can't be reached in the treatment, additional use restrictions may be helpful in minimizing the risks of direct transmission via food and feed to man and animals. Since use restrictions are ineffective in minimizing the environmental risks and very limited in avoiding the phytohygienic risks, safe treatment must have the priority before applying the tool of use restrictions.

Keywords: hygienic safety, HACCP, biowastes, sewage sludge, manure, process validation

INTRODUCTION

Recycling of biological wastes by aerobic or anaerobic biotechnological treatment as well as by physical or chemical stabilizing treatment mainly results in the production of organic fertilizers, soil improvers, growth media or comparative products deemed to be used mainly in agriculture or gardening. Municipal wastes, animal by-products, sewage sludge and other organic sludges may contain pathogens of different nature being infectious for several species of animals and plants as well as for humans. Depending upon the type on the type of pathogen and on the type of wastes and residuals, the epidemiological importance for possibly exposed populations of animals,

humans and plants differs depending on origin, storage and treatment of the relevant materials and types of organisms causing the risks (Table 1).

Strategies for minimizing the risks

Hazard Analysis Critical Control Point (HACCP) quality assurance systems are used internationally in the food industry to ensure product quality standards are met and with some modifications, it is part of EU legislation on animal by-products and of several EFSA opinions in 2005. However, the system can be adapted to the treatment of other organic wastes and residuals including sewage sludge and energy crops, particularly to guarantee hygienic standards. The three key actions to be taken to establish an effective HACCP concept are; process validation, process supervision, and product supervision. In the case when a remaining risk has been identified, restrictions on usage may be the fourth measure to be taken. First the risks related to the amount and type of pathogens as well as microorganisms with undesired properties to be attended in the raw material must be considered as well as the intended field of application for the final product, because safe utilization can avoid certain hygienic risks. The initial stage of establishing a HACCP system in the treatment plant itself is to undertake a hazard analysis, which identifies points in the treatment process, which are critical to delivering the final product standards. At these points, the relevant process parameters related to the inactivation of pathogens shall be continuously measured such as temperature, time, concentration, pH value etc. For those Critical Control Points (CCPs) control data for supervision of the safe inactivation of the relevant pathogens can only be fixed reliable by a validation procedure testing the degree of inactivation of such pathogens using representative test organisms in an experimental approach (process validation). Once validated, the HACCP system operating limits are set, and by operating the system within those limits the end product quality is assured. This means that end product testing which is always critical due to the inability to define a representative sample size and number as well as due to of the inhomogeneity of the bulk material, can be reduced to a level which limits microbiological end-product control to the monitoring of a reasonable amount of samples. For each process a contingency programme is required, which details the plan of action if any CCP goes outside its limits and ensures that failed product cannot contaminate assured product.

Table 1. Epidemiological importance of organic wastes and residuals as well as of the resulting fertilizer during transport, treatment and utilization

A.	**Direct transmission to farm animals**
	➪ Contamination of meadows
	➪ Introduction of pathogens by storage and processing close to susceptible animals
	➪ Aerogenic transmission by spreading the materials onto farm land
B.	**Direct transmission to humans**
	➪ Handling of contaminated fertilizers in the household
	➪ Occupational exposure to contaminated products
	➪ Accidental transmission to immuno-compromised persons
C.	**Indirect transmission to farm animals**
	➪ Via feed from contaminated sites
	➪ Via living vectors
D.	**Indirect transmission to humans**
	➪ Via introduction of zoonotic agents into the food-chain
	➪ Via food contaminated by living vectors

E.	**Introduction into the environment**
	➪ Generation of carriers in the fauna
	➪ Introduction of organisms with undesired properties into the biocoenosis and persistence in soil and water

Process Validation is a key tool

The capability of a process to inactivate pathogens causing raw-material dependent risks cannot be judged by analysis of presence or absence of indicators (Bacterial, viral, fungal or parasitic) in the final product. Absence of all or one of the mentioned pathogens or indicators in the final product may be caused by several reasons: They may not be present at this time in the raw material, or they are present in the raw material but in a low count (less than 5 log), the recovery of the involved pathogens may be insufficient due to ineffective enrichment or resuscitation procedures (bacteria) or there may be a failure of isolation due to effects of the complex matrix (viruses). Therefore validation strategies must be followed taking two basic approaches into account. The easiest way is to perform an input-output analysis measuring the inactivation rate of one or more organisms present in the raw material during the treatment process, the other is direct validation of the treatment process by exposing test organisms with defined patterns of thermo- and/or chemo resistance for assessment of the inactivation rate. Validating a process by input-output analysis of a certain indicator is generally possible but under practical conditions of limited importance. In most cases, depending on the microbiological properties of the input materials processed, other strategies must be followed, e.g. process validation with one or more representative test–organism. Either if the thermophilic process itself or if a thermal treatment shall provide an inactivation of pathogens belonging to the indicated level of thermo- and chemo resistance representative test-organisms must be exposed in a similar matrix as treated in a suitable test-body in a defined validation experiment. The relevant process parameters must be recorded during the exposure in order to define the technical conditions to be maintained for effective inactivation according to the results of the survival experiments.

The question of how validation shall be performed and what test containment system can be applied is not easy to answer. In biogas plants two main types of test containments may be applied depending on the test organisms. For those test organisms which can be retained in a test containment system filled with liquid by a membrane filter like bacteria, fungi and parasites type 1 test containments could be used (Rapp, 1995). Exposure of viruses to a process requires a different test containment systems. In such a type 2 system the virus material is adsorbed to a special filter material and released after exposure by desorption due to washing with a special solution according to Traub et al. (1986) and Hoferer, (2002). In composting different approaches are described for bacteria, because a representative amount of raw material can directly be contaminated with the test strain, put into textile sacks protected by a perforated metal basket from mechanical destruction Viruses may be exposed also surrounded by the material deemed to be composted, but in a type 2 test containment system as described above.

Process validation with vegetative Bacteria

Several different test organisms had been discussed in the European context, the most promising are: *Escherichia coli, Salmonella Senftenberg* W775 W, H2S negative and *Enterococci*, e.g. *Enterococcus faecalis*. Table 2 gives some thermoresistance data in a matrix representative in co-fermentation of liquid manure together with catering wastes.

Every of the indicated test organisms have advantages and disadvantages. Escherichia coli is generally less fit in biogas-plants and in composting as *Salmonella Senftenberg* W775 H2S negative and there is no scientific background for the selection of a certain test strain. Moreover enrichment from environmental samples is not as sensitive and effective as for Salmonella.

Table 2. Thermoresistance data (maximal D – values) for selected vegetative bacteria in co-digestion of slurry with catering wastes

SPECIES	TEMPERATURE	50 °C	50 °C	55 °C	55 °C
	Slurry	Pig	Cattle	Pig	Cattle
E. coli		0,43 h	0,40 h	0,08 h	0,03 h
S. Senftenberg W 775, H2S negative		0,60 h	0,53 h	0,11 h	0,06 h
Enterococcus faecium		7.48 h	11,2 h	1,7 h	1,64 h

Even when the test containments are hermetically sealed, accidental contamination from outside cannot totally be excluded under mechanical action in the reactor. In this case, differentiation from contaminants with other E. coli of faecal origin is not so easy to achieve. This may be overcome by the use of mutants of E. coli, but K12 mutants are less fit than field strains. The use of *Salmonella Senftenberg* W775, H2S negative has some advantages. First a reliable quantitative enrichment, also from a contaminated test body, is easy to achieve. Methods are described in CEN WI 308. 049 1–3. *Salmonella* are really representative test organisms with epidemiological importance and not a surrogate. The thermo resistant mutant (H2S negative) used for validation allow a easy differentiation from native Salmonellas (H2S negative) and there are many data available from various survival studies and validation experiments. But there are also some disadvantages, one of the often raised points is, that according to the nomenclature it is still regarded as a pathogen this seems to limit the application (e.g. in Scandinavian countries). For the thermophilic biogas process does not cover the relevant viral pathogens totally in resistance. Hoferer (2002).

Finally *Enterococci* have also some advantages, especially as there are fewer concerns with regard to pathogenicity. As for *Salmonella Senftenberg* there is also a lot of existing data for *Enterococcus faecalis* concerning validation of thermal processes, but mainly in other application fields (hospital hygiene). Since it is more resistant than Salmonella, a quantitative analysis of the results from the validation of pasteurization units is possible, while Salmonella are inactivated too rapidly and bacterial spores are too resistant. The application of *Enterococci* for this purpose also has some disadvantages. First the quantitative enrichment is less effective than in Salmonella, if contaminant flora shall be excluded. In this case contamination from the substrate cannot be detected in an easy and reliable way. Finally it must be kept in mind, since *Enterococci* do not have any epidemiological importance and since they are more chemo- and thermo resistant than most relevant pathogens in this field their application may set a much too high barrier for passing such a validation in certain situations. This means, that the application of test organisms must be strictly related to the process to be evaluated. Therefore if only a process of thermal inactivation like a pasteurization-unit is being validated, *Enterococcus faecalis* is the suitable test organism. If a thermophilic aerobic or anaerobic process shall be validated, it is more realistic to use the above characterized strain of Salmonella Senftenberg, because Enterococci will be a too hard criteria for this purpose, since its chemo-resistance differs substantially from most of the relevant pathogens.

Validation with thermoresistant viruses

In certain epidemiological situation e.g. if animal by-products shall be processed validation with thermoresistant viruses is necessary. It is known from comparative heat inactivation studies, that the Bovine Parvo Virus (BPV)is much more resistant than enteroviruses and will survive treatment at 70°C for 60 min (Stöcklein, 2005). Since recently it was stated by Emmoth et al. (2004) that heating of animal by-products to 70°C for 60 min is not enough for the inactivation of circoviruses and it had been demonstrated that the plant pathogenic tobacco mosaic virus withstands such treatment without any significant reduction, there is a necessity to validate the treatment if such viruses may be present in involved materials. The following viruses may be used in principle as test-viruses in process validation: Parvovirus (bovine, feline) or Circovirus (porcine, avian). Limited results are available concerning the application of thermoresistant viruses in validation procedures. Most data is available with the application of bovine parvovirus in validation of composting and biogas plants. Table 3 gives some D-values in mesophilic and thermophilic cofermentation units. The given data are demonstrating, that not only the temperature but also the type of substrate is influencing their survival.

Process validation with parasites

Due to their lower thermoresistance most parasites eggs and oocysts give no additional information in the validation of thermal processes. But in validation of chemical treatment they may be useful with certain substrates. The exposure techniques are the same as for bacteria. Test organisms which may be used are eggs of *Ascaris suum.* Oocysts of *Cryptosporidium parvum* may also be used, but there is no reliable technique available to judge definitely over inactivation, viability and infectivity, Mayer (2001).

Table 3. Decimal destruction rates as Dt – values in hours (T90 – values) found for several viruses in co-digestion (catering waste and slurry) at different temperatures according to Hoferer (2002)

Dt in hours	Temperature	30°C	30°C	30°C	35°C	50°C	50°C	55°C	55°C
Type of virus	Type of slurry	CS	PS	CS*	PS	CS	PS	CS	PS
ECBO	A	43,44	24,72	25,20	17,36	0,61	0,12	0,24	0,07
ERV	A	34,08	25,92	N.	N.	0,96	0,72	0,54	0,20
Polio	A	N.	32,16	N.	N.	0,63	0,18	0,07	0,03
BPV	A	N.	N.	180,24	N.	20,41 10,48	14,27	4,67	5,47

N = not investigated
* = long time stored pig slurry without catering wastes
A = test organisms absorbed to membranes
PS = pig slurry
CS = cattle slurry

Process validation with bacterial spores

Process validation with bacterial spores is only useful if sterilization processes have to be evaluated. The exposure techniques have to be different from those described above. CEN TC 102 deals with such subjects, the relevant standard is EN 556. Biotechnological treatment will not inactivate bacterial spores neither of Bacillus species nor of Clostridia, as well as pasteurization at

70°C will fail for this purpose (Stöcklein, 2005). Test organisms which may be used in this context are spores of *Bacillus stearothermophilus, Bacillus subtilis* and *Clostridium sporogenes.*

Process validation with prions

If processes deemed to treat category 1 material according to EU regulation 1774 /2002 need to be validated, this have to be done with PrPres. The most convenient way to do this is validation with the hamster adapted strain like 263 K which can be propagated to high titres in a hamster brain and rapidly titrated back after exposure in a bioassay within 90 days. But it must be kept in mind, that BSE-PrPres will not be covered by all scrapie strains in heat-resistance (FAIR, 2001). Comparative studies are needed between representative BSE-strains and the scrapie-strain 263 K. Until then it may be discussed, if a hypothetical six log reduction of strain 263 K in a validation procedure may be regarded as sufficient to cover a five log reduction of a resistant BSE-strain under practical conditions.

Process Supervision

The critical control points identified in the basic analysis and in the validation of the treatment have to be continuously supervised, the relevant data have to be recorded and filed for at least 2 years. There are two types of control points, those which had been identified in the basic analysis of the flow of material in the plant and which are mainly of an organisational nature on the one and those in which technical parameters have to be monitored which are directly related to the treatment process and the associated results of the validation procedure. The organisational control points are mainly related to any form of documentation necessary to assure the keeping of standard operation procedures including the fixation of responsibilities which are common in any HACCP – concept.

With relation to process supervision the technical parameters to be kept are of primary importance. Those parameters are in general dependent on the type of treatment procedure. This means that, for example in a composting process, very simple parameters have to be measured like exposure time related to temperature, moisture and frequency of turning the material, while more complicated technical parameters have to be recorded as in animal by-product treatment like feed screw revolutions per minute (rev. /min.), electric power (amps at given voltage), evaporation/condensation rate, number of pump strokes per unit time. All measuring and monitoring equipment must be calibrated at least once a year. The definition of such control points and the values to be kept are basically related to the results of process validation.

Product Supervision

As mentioned above, the investigation of the final product in order to detect every pathogen which may be present in the material is impossible, therefore representative indicator organisms have to be determined from the point of view of human and animal health as well as for the purpose of safe plant-breeding and production. Those indicator organisms must fulfil several requirements: they have to be present with a high probability in the raw materials, the transmission via the final product must be a factor in epidemiology, the indicator should not be involved in the biotechnological process itself, the indicator should not be an organism which is generally present in soil and soil related materials and the method for isolation and identification must be simple, definitely and reliable if applied to a substrate with a complex microbiological matrix such as compost or digested material.

With respect to public health and veterinary requirements several indicators and parameters are in discussion: *Salmonella enterica, Enterococci* (*Streptococci* of group E), *Staphylococcus aureus, Enterobacteriaceae, Escherichia coli,Campylobacter, Yersinia spp, Listeria spp, Clostridium perfringens,* Sulfite reducing *Clostridia,* Enteroviruses, Rotavirus, eggs of nematodes and larvae of nematodes Materials coming out of processes such as composting or anaerobic digestion are products of a microbial degradation and the knowledge about the microbiological ecology of such materials is still limited. Consequently it is important that, if analysis methods based on clinical microbiology or drinking water examination are used for isolation and identification, a careful validation in combination with all the involved sample matrices is essential.

The variety of species present in environmental sample and in such complex matrices as compost by far exceeds the limited number of species to be taken into account in excreta as well as in body fluids. The variability in species in compost-like materials is very high and not yet fully understood. Moreover, microbial parameters which are used in the field of water hygiene and food inspection are not applicable to substrates like compost or sludges from anaerobic digestion, because most of those indicators belong to the indigenous flora of agricultural soils (Böhm, 1995). It must be taken into account, that methods used in clinical microbiology and in drinking water supervision will often fail if applied to complex matrices like compost or digested sludge. Same applies for the selection of so called indicators. Since most of the materials are of faecal origin, faecal indicators have to be present in the material. The intended field of use has so to be taken into account in this context, therefore the exclusion of organisms which generally may be found in normal soils makes no sense for a substrate and fertilizer as e.g. compost. This means that the following microbial parameters are, with some exceptions in certain situations, inappropriate: *Staphylococcus aureus, Enterobacteriaceae, Clostridium perfringens,* sulphite reducing *Clostridia* and *Listeria.*

One parameter which seems to be very useful and reliable in this connection is the absence or presence of *Salmonella.* There is a high probability of finding *Salmonella* (at a range of levels) in fresh biowastes or untreated sewage sludge. Since it is known that the probability of identifying a positive sample is basically related to the amount of investigated material a compromise between feasibility and reliability has to be found. It is proposed to take 50 g or 100 g (2x50 g) of material to determine presence or absence of *Salmonella.* The approach of the European animal by-product regulations is to use only 25 g of material as has been the practice 15 years ago (ATV 1988) and gives less sensitive performance data as if 50g of material were to be used. Some other parameters are still in discussion with respect to sewage sludge treatment and composting in the framework of EU – directives. *Enterococci* for example cannot be used as indicator in the examination of compost and compost related products, but for the thermophilic anaerobic treatment in biogas plants as well as for pure thermal treatment they are very valuable (Bendixen, 1999). For E. coli, Campylobacter and *Yersinia* beside the lack of reliable re-isolation techniques it must be stated that their thermal resistance and with minor exceptions chemo-resistance is lower than that of *Salmonella*. This means it will make no sense if they are used as additional microbial parameters for describing a hygienically safe product. Enteroviruses are generally present in sludge of faecal origin but not regularly in sludges coming from other sources. In principle Enteroviruses may be used as an additional indicator but the re-isolation procedures are, as for all viruses from environmental samples, both labour and cost intensive. Their resistance in the involved treatment processes is not higher than that of Salmonella, this means, that the additional information resulting from using this indicator organisms are of little value. The same applies for rotavirus;

even it is of special environmental importance according to Metzler et al. (1996) and Pesaro et al. (1999).

The question whether nematodes or nematode eggs are a useful indicator in this connection is not easy to answer. With respect to nematodes pathogenic for man and/or animals the experience shows, that even eggs of *Ascaris suum* are less thermo resistant than *Salmonella*, but behave differently in chemical treatment, this means that if *Salmonella* would have not survived e.g. the (thermal) composting process *Ascaris* eggs and with them all other nematodes eggs would not have done either. This does not apply for treatment with slaked lime or long-term storage. This means that *Ascaris* eggs will not be a necessary indicator in all processes in which the thermal effect is the predominant one but they will give valuable additional information if used in the supervision of all other treatment processes. Nematodes may also be an indicator for insufficient storage conditions for a final product like compost by which plant pathogenic nematodes may have invaded the material. In order to identify this situation eggs or larvae of such species have to be properly identified. This requires special expertise, which is generally not available in most of the relevant laboratories. This means that a general parameter defined as free of nematode eggs and/or larvae" will not be easy to realize in this connection. Another situation in which the investigation of the final products for the presence of nematode eggs makes sense is in co-digestion with liquid manure or sewage sludge if this feeding material had not been heated before entering the reactor. No plant pathogenic virus, fungus or bacterium has been found so far which is comparable in importance to Salmonella for the above-mentioned purpose. The only indicator, which is widely distributed in biological wastes from households and in wastewater, is tomato seed. Even this indicator will not totally cover all requirements, additionally assessment of all reproducible parts of plant materials from biowastes have also to be taken into account. Therefore it seems reasonable and feasible to define the term "phytohygienic safety" of the product as done in the German Biowaste Ordinance: "The final product may not contain more than two tomato seeds per litre product that are capable of germinating and/or reproducible plants parts". A suitable test-method is described by Bundesgütegemeinschaft Kompost (1994).

Use Restrictions

Restriction in the use of fertilizers and substrates resulting from biotechnological waste-treatment should either prevent introduction of undesired chemical residuals by contaminated crops into the food chain of direct transmission of pathogens to susceptible animals via feed. This has been practised in the past especially with sewage sludge. Such a strategy alone does not prevent the environmental risks or introduction of pathogens into vector populations, which will lead to indirect transmission cycles. Several authors have given examples how birds can become carriers of *Salmonella* (Hellmann 1977). One of the sources of infection in sea gulls has been found to be a sewage treatment plants. The further ways of introduction of a certain lysotype of Salmonella enteritidis could be demonstrated by Köhler (1993). He identified the waste delivered from West Berlin to a waste disposal site in the former GDR and followed the introduction of this pathogen via birds into the chicken populations and finally to humans via products containing eggs. (Williams et al., 1977) as well as several other authors like Coulsen et al. (1983), Mayr (1983) described the importance of vectors in the transmission of *Salmonella* to farm animals and humans. Foster and Spector (1995) described specific molecular mechanisms responsible for the ability of *Salmonella* to survive the environmental stress. This means even if the fertilizers containing pathogens are immediately ploughed into the soil or injected by special devices they may generate carriers (e.g. sea-gulls attracted by ploughing) or prolonged survival in sub surface

soil layers. Thus restrictions in use are a tool with limited effects from the point of view of epidemiology and should be avoided if possible and feasible. Moreover concerning plant pathogens and seeds this strategy is ineffective if the products are to be used in agriculture.

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FAECAL MICROORGANISMS IN RUNOFF FROM CATTLE AND HORSE FARMS – QUANTIFICATION AND MITIGATION

Uusi-Kämppä, J.[1], Hyvärinen, M.[1], Kuisma, M.[2], Nykänen, A.[2], Jansson, H.[1], Jansson, H.[3] and Närvänen, A,[1]

[1] MTT Agrifood Research Finland, Plant Production Research, E-talo, 31600 Jokioinen, Finland; [2] MTT Agrifood Research Finland, Plant Production Research, Lönnrotinkatu 3,50100 Mikkeli, Finland; [3] MTT Agrifood Research Finland, Animal Production Research, Equine Research, Varsanojantie 63, 32100 Ypäjä, Finland

SUMMARY

Nutrient runoff from agricultural sources to watercourses has been decreased in different ways during last decades. Indeed, until recent years, pathogen transmission via agricultural runoff waters has been under less concern. According our studies levels of faecal microbes can be extremely high (e.g. 10^3–10^6 colony forming units of faecal coliforms in 100 mL) in runoff waters from exercising areas used by cattle or horses while the limit of good bathing water is <500 CFU/100 mL. The transfer of pathogens can be reduced e.g. by removing manure from lots, establishing of buffer zones and purification of waters.

Keywords: faecal indicator bacteria, faecal coliforms, horse paddocks, livestock, exercise yards, runoff, transport, water hygiene, buffer zones

INTRODUCTION

Along with the recent growth in the size of cattle farms, their concentration in certain areas and increasing number of horses close to urban areas, hygiene problems with manure management and agricultural runoff are growing in Finland. Runoff waters from fields with slurry applied on the surface, yards used as exercising areas for cattle, and equine paddocks may contain high numbers of faecal microbes which can act as vectors of disease transmission from agricultural areas. This paper consists of levels of faecal coliforms, enterococci, presumptive *E. coli*, presumptive coliforms and sulfite-reducing clostridia which are used as indicators of potential microbial pollution in water, and how to mitigate pathogens from runoff water in cattle and horse farms. Results are compared with previous studies done at the MTT Agrifood Research Finland.

MATERIALS AND METHODS

Study sites were 1) four exercise yards (covered with asphalt, woodchips or sand) and two open feedlots used by cattle, 2) a clay field with slurry applied on the surface and direct sowing, and 3) four horse paddocks. Because there was high variation in stocking rates (SR) and time animals spend in the yards (d), we calculated loading intensity (LI) according to the following formulas:

(1) LI=SRd, where

LI=loading intensity (livestock unit day/hectare year; LSUday/ha yr),

(1a) SR=stocking rate=LSU/A, where
LSU=livestock unit (1 dairy cow or 1 horse = 1 LSU, 1 heifer or 1 bull less than 2-yr-old = 0.6 LSU), A=area of a lot (ha)

(1b) d=tn/24, where
t=exercising time/day (h), and n=number of exercising days/year.

Water samples were collected from sumps or drainage water wells adjacent to the exercise yards. Plant-soil-samples (10-cm-deep layers) were taken from (1) a clay field with a slurry application of 20 t ha^{-1}, (2) buffer zones (BZ) between the clay field and a ditch, and (3) four horse paddocks. The plant-soil-samples were used in a rain simulation study (20 mm h^{-1}) for 2 hours and surface water samples were collected during the rainfall simulation. The water samples were filtered for faecal coliforms, enterococci and both presumptive *E. coli* and coliforms through Millipore 0.45 µm and for sulfite-reducing clostridia through Millipore 0.22 µm filters. Faecal coliforms (SFS 4088), enterococci (SFS-EN ISO 7899-2) and *E. coli* were then cultivated on mFC agar (Difco), KF Streptococcuc agar (Difco) and Harlequin *E.coli*/coliform medium (LabM), respectively. On the Harlequin medium, blue-purple colonies were counted as presumptive *E. coli,* and all blue-purple and magenta colonies were counted as presumptive coliforms. Sulfite-reducing clostridia were determined by SFS-EN 26461-2 and incubated in an Oxoid anaerobic jar. Bacteria counts were expressed as geometric means of colony forming units (CFU) per 100 mL of water.

RESULTS

Exercise yards and outdoor feedlots

Levels of faecal coliforms, enterococci and sulfite-reducing clostridia were the highest in surface runoff from asphalt yards (Table 1). Although some faecal micro-organisms were retained by woodchips on yard surface or by sand yards, the levels were still high in runoff. Soil filters used for cleaning runoff waters from asphalt yards were quite efficient at first but their purification capacity dropped if the suspended material was not removed from the water before purification (Uusi-Kämppä & Heinonen-Tanski 2000). After one year from the introduction of a 0.8-m-deep sand filter, faecal coliform levels from the asphalt yard runoff (7.0×10^6 CFU/100 mL) were decreased to outgoing runoff (3.6×10^5 cfu/100 mL). For enterococci and sulfite-reducing clostridia 10–100-fold lower values were obtained after the filtration of yard runoff by the 0.8-m-deep sand layer. Soil or sand floor of yards decreased bacteria levels compared with asphalt yards (Table 1). Also at a forested feedlot (2200 LSUday/ha yr), levels of faecal coliforms and sulfite reducing clostridia in a ditch water were small (220 and 44 CFU/100 mL, respectively; Uusi-Kämppä et al. 2006).

Table 1. Stocking rate, loading intensity, and geometric means of faecal indicator levels in runoff water from exercise yards and forested feedlots. (Number of samples is presented in parentheses)

Exercise yard /Feedlot	Stocking rate (LSU/ha)	Loading intensity (LSU day / ha yr)	Faecal coliforms (CFU/100 ml)	Enterococci (CFU/100 ml)	Sulfite- reducing clostridia (CFU/100 ml)
Asphalt yard (4)[(a)]	370–670	17,000–30,000	4.8×10^6	3.8×10^6	4.6×10^3
Yard covered with woodchips (14)[(b)]	620–1100	28,000–50,000	5.0×10^4	7.8×10^3	150
Sand yard (3)[(c)]	330	45,000	7.2×10^4	2.3×10^4	120
Asphalt yard (1)[(d)]	770	30,000	7.0×10^6	3.1×10^6	1.1×10^4
Asphalt lot covered with woodchips (2)[(e)]	140	30,000	5.9×10^4	1.9×10^4	2.8×10^3
Forested feedlot (3)[(f)]	77	17,000	1.1×10^4	3.8×10^3	250

[(a), (b)] Established in 2000, studied between 2001 and 2006, ca 3 h exercising in the yard daily for 12 months/yr (Uusi-Kämppä et al. 2006)
[(c)] Established in 2004, studied between 2005 and 2006, 24 h exercising in the yard daily for 4.5 months/yr
[(d)] Study in June 2000, ca 2 h exercising in the yard daily for 7.5 months/yr (Uusi-Kämppä & Heinonen-Tanski 2000)
[(e)] Study in June 2000, rearing daily (24 h) for 7.5 months/yr (Uusi-Kämppä & Heinonen-Tanski 2000)
[(f)] Established in 1995, rearing daily (24 h) for 7.5 months/yr, studied percolation water at 0.3 m, near feeding fences in 2000 (Uusi-Kämppä & Heinonen-Tanski 2000)

Field runoff and surface runoff under rainfall simulation

Levels of faecal coliforms were high in the surface runoff from a pasture in summer 2004 and 2005 decreasing quite soon in the cool autumn weather. For the faecal coliforms, 2–20-fold lower values were generally obtained in the surface runoff from pasture with a 10-m-wide cut grassBZ than from the pasture without a BZ (No-BZ). In the USA, Frantz et al. (2003) presented that a BZ system was able to reduce pathogen indicator levels from dairy wastewater by 100 to 10,000-fold. On an uncut vegetated BZ, the decrease of faecal coliform levels was less than on the grassBZ and sometimes the levels also might increase compared with the no-BZ. A reason for the increase of faecal coliforms might be voles living on the vegetated BZ. Sometimes they were found in the collected runoff water which was sub-sampled.

At a rainfall simulation study, the levels of enterococci, *E. coli*, coliforms and sulfite-reducing clostridia were high in surface runoff from 10-cm-deep plant-soil-systems two weeks after cattle slurry application (20 t/ha) (Table 2). The levels of hygiene indicators in surface runoff from BZs (not amended with slurry) were generally 100–1000-fold smaller compared to the levels of slurry-applied field runoff. In our study, levels of sulfite-reducing clostridia decreased below the detection limit in 6 weeks from the slurry application. The other indicator levels also decreased little by little during 6 weeks and especially after freezing the plant-soil-samples. In an earlier study, where slurry was applied on the surface of grass field with a 10-m-wide unmanaged BZ, the levels of total coliforms, enterococci and sulfite-reducing clostridia were in surface runoff water similar (1.9×10^4, 4.8×10^3 and 1.5×10^3 CFU/100 ml, respectively) as presented here (Heinonen-Tanski & Uusi-Kämppä 2001).

Table 2. Geometric means for numbers of hygiene indicators in runoff water from plant-soil-samples under a rainfall simulation two weeks after a slurry application and direct sowing in September 2006. Slurry was applied on the field and on the No-BZ

Treatment	Enterococci (CFU/100 mL)	*E. coli* (CFU/100 mL)	Coliforms (CFU/100 mL)	Sulfite-reducing clostridia (CFU/100 mL)
Field (n=6)	2.9×10^6	5.2×10^4	1.2×10^6	710
No-BZ (n=2)	8.6×10^5	2.5×10^4	2.1×10^5	230
Cut grassBZ (n=2)	3.8×10^3	91	5.8×10^3	13
Uncut vegetated BZ (n=2)	1.4×10^5	50	3.3×10^4	<1

Equine paddocks

Under a rainfall simulation, the levels of *E. coli* and coliforms were very high in surface runoff from soil samples taken from horse paddocks (Table 3). The samples were collected after a warm and dry summer in August 2006. The highest levels were measured from the paddocks 1 and 2 where the manure was not removed from the surface. Numbers of *E. coli* were also smaller in runoff from paddocks covered with woodchips or established on a clay soil. Addition of 10 t Ca $(OH)_2$ ha^{-1} to the surface of the soil before rainfall simulation decreased coliform levels in the runoff (Table 3). In drainage water from the paddock 3, the levels of *E. coli*, coliforms and faecal coliforms were also rather high (1.3×10^4, 5.6×10^4 and 2.3×10^4 and CFU/100 mL, respectively) in the end of October 2006. Levels of sulfite-reducing clostridia were generally under the detection limit in the horse paddock runoff. According to Airaksinen et al. (2007) the levels of faecal coliforms, enterococci and sulfite-reducing clostridia were 5–4700, 18–2200 and <100 CFU/100 mL in surface runoff from two horse paddocks (37.5 animals/ha) in eastern Finland.

Table 3. Loading and geometric means for numbers of hygiene indicators in runoff water from paddock soil samples under a rainfall simulation in August 2006. (n=number of samples)

Place / Treatment	Loading (LSU day/ha yr)	*E. coli* (CFU/100 mL)	Coliforms (CFU/100 mL)
Paddock 1 (rubble cover and manure; n=2)	4400	3.2×10^7	7.8×10^7
Paddock 1 (rubble cover; n=2)	4400	1.8×10^5	9.2×10^5
Paddock 2 (sand cover with manure; n=2)	7000	1.4×10^5	1.4×10^6
Paddock 2 (sand cover; n=2)	7000	1.1×10^5	1.1×10^6
Paddock 2 + 10 t $Ca(OH)_2$ ha^{-1} (n=1)	7000	<5	<5
Paddock 3 (covered with woodchips; n=2)	6000	9.5×10^4	2.6×10^6
Paddock 4 (clay soil; n=2)	8200	2.0×10^4	4.0×10^5

Paddock 1: 710 m^2, 1–2 horses for 4 periods between 1.5 and 4.5 hours daily since 2005
Paddock 2: 520 m^2, 1–3 horses for 4 periods between 1.5 and 4.5 hours daily since 2003
Paddock 3: 1900 m^2, 7 horses for 11 hours daily since 2005
Paddock 4: 800 m^2, 4 horses for 11 hours daily since 2001

A sedimentation pond with an addition of ferric sulphate was used for phosphorus retention from runoff waters from equine areas at Ypäjä, Southwest Finland (Närvänen et al. 2006). This system also seemed to decrease numbers of hygienic indicators in outgoing water compared with incoming runoff (Table 4).

CONCLUSIONS

Most runoff waters contaminated by faeces would not fulfil the requirements for bathing waters, where faecal coliforms should be less than 500 CFU/100 mL and enterococci less than 200 CFU/100 mL. The feeding and drinking areas were the most contaminated areas both on feedlots (Uusi-Kämppä & Heinonen-Tanski 2000) and horse paddocks (Airaksinen et al. 2007). Exercise yards and horse paddocks should be established so that the risks of pathogen transmission to waters can be controlled. Cleaning of paddocks, establishing buffer zones between source areas and waterways, and e.g. addition of calcium hydroxide to critical source areas decrease faecal microorganisms in runoff water. Filters blocked with suspended materials were not able to reduce levels of neither faecal microorganisms nor other pollutants from the yard runoff. More research is, however, needed to solve hygiene problems in agricultural source areas.

Table 4. Numbers of hygiene indicators in grab samples of incoming runoff water from equine areas and outgoing water purified by ferric sulphate in a sedimentation pond

Date/incoming/outgoing	Faecal coliforms (CFU/100 mL)	Enterococci (CFU/100 mL)	*E. coli* (CFU/100 mL)	Coliforms (CFU/100 mL)
15 Nov 2005 / incoming	5.0×10^4	9.4×10^3	n.a.	n.a.
15 Nov 2005 / outgoing	3.2×10^4	8.8×10^3	n.a.	n.a.
25 Oct 2006 / incoming	7.2×10^4	n.a.	2.7×10^3	4.4×10^4
25 Oct 2006 / outgoing	1.3×10^3	n.a.	<500	<500
7 Dec 2006 / incoming	n.a.	400	600[(a)]	n.a
7 Dec 2006 / outgoing	n.a.	0	0[(a)]	n.a
14 March 2007 /incoming	n.s.	n.s.	n.s.	n.s.
14 March 2007 /outgoing	n.a.	69	0	130

n.a.=not analysed, n.s.=not sampled
[(a)]=used the standard of SFS 3016 and confirmed by indole test

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SEASONAL CHANGES OF ZOONOTIC AGENTS PRESENCE IN DAIRY MANURE OF MODERN AND TRADITIONAL FARMS

Santorum, P.[1], García, R.[1] and Fernández, B.[2]

[1] Centro de Investigación y Formación Agrarias, Consejería de Ganadería, Agricultura y Pesca del Gobierno de Cantabria, C/ Héroes 2 Mayo, 27. 39600 Muriedas (Cantabria, Spain); [2] Servicio de Agricultura y Diversificación Rural, Consejería de Ganadería, Agricultura y Pesca del Gobierno de Cantabria, Edificio Europa, C/ Gutierrez Solana, s/n. 39001 Santander (Cantabria, Spain)

SUMMARY

Two clusters were investigated, one with upscaled waste facilities and another with traditional ones. The microorganisms looked for were zoonotic agents (protozoa, bacteria and viruses). Upscaled facilities produced more diluted and more saline manures than traditional ones. Results showed summer and spring as the seasons when the higher number of manures presented zoonotic agents, followed by autumn. The presence of each zoonotic agent depended on season and on farm type. Total aerobic bacteria and faecal coliform counts varied in upscaled farms and faecal streptococcus counts in traditional farms, depending on season.

Keywords: dairy, zoonotic agents, manure, wastes, spreading, indicators

OBJECTIVE OF THE WORK

Organic amendment of soil is the widest reuse practice in manure management. Current practices in most of dairy farms in Cantabria (Northern Spain) involve spreading of all the manure produced and stored as fertilizer, without composting or another treatment. In the present economical, geographical and structural conditions in this region, dairy farms' size is increasing, just as volume of manure does. Product price based on quality standards and related legislation forced farmers to install or upscale the milking machinery. Taking into account that this updating is not always accompanied by waste stores upscaling, very different types of farms can be found. No updating of waste facilities or machinery parallel to the rise in waste volume makes the frequency of waste spreading becomes higher. Consequently, risk in pathogens' dissemination could be increased and should be evaluated.

MATERIAL AND METHODS

We have grouped dairy farms by clustering in four types, according to farm size, manure store type and manure spreading system. The second cluster included middle size farms that have constructed covered stores and own the tanker. The third one groups small farms with uncovered tanks or piles to storage manure and that use manual spreading, pumping or solid spreaders (Santorum, 2007). The two extreme situations were investigated in relation to health risk: whole

adaptation of waste management facilities and machinery (second cluster) and traditional waste management (third cluster). The updated farms' size fluctuates between 100.000 and 500.000 kg of milk production per year, while the traditional farms produce less than 100.000 kg. Five facilities of each cluster were selected in order to evaluate the physicochemical composition and the number of pathogens present in manure sampled in spring, summer and autumn.

The physicochemical composition of the manures to be fertilized was ascertained regarding to pH, conductivity and dry matter. The first and the second parameters were measured by pH meter equipped with a salinity meter. Dry matter was calculated as the loss of weight until it is constant, while subsamples were maintained in drying oven at 75ºC for at least 48 hours. Flow injection analysis provided ammonia content in manure subsamples.

The detection of protozoan cysts was performed by a modification of Ziehl-Neelsen acid-fast method. The separation included centrifugation followed by flotation in saline solution (d=1.22) and the identification of the cysts was confirmed after staining *C. parvum* oocysts with Kingyou kit (MAIM) and with iodine lugol for *Giardia* cysts. The presence of typical cysts was assessed in 10 randomly selected fields at 400x and 1000x magnification for *C. parvum* oocysts and in the whole cover slip at 400x for *Giardia* cysts.

In the case of *Salmonella* spp., resuscitation and selective media cited in UNE-EN ISO 6579:2002 were choosen. *L. monocytogenes* detection was achieved following UNE-EN ISO 11290-1:1996/Amd. 1:2004. Nutrient broth was employed for recovering *Campylobacter* spp, to which lamb blood (Biomerieux) and Preston Campylobacter selective supplement were added. Loops of liquid cultures were streaked on the selective medium for *Campylobacter* spp. (recovering broth plus agar). Further confirmation of the typical colonies was achieved with API Campy (Biomerieux) and with oxidase tests and Gram staining. Two bacteria were isolated directly from manure subsamples on agar selective media: *Mycobacterium* spp. on MGIT agar, and *Brucella* spp. on Farrell's medium (Oxoid). All culture media, unless otherwise stated, were from Merck. Rotavirus and coronavirus were diagnosed using ELISA kit developed by Institut Pourquier (ref. P00603). When all the incidences in manures were grouped, the number of manures that presented at least one pathogen (positive manures) was estimated for each season and cluster.

Total aerobic bacteria as well as bacterial indicators that are linked to faecal contamination were enumerated (UFC/g). Manure subsamples were serially diluted in Ringer solution and volumes of 1 ml were mixed with melted agar. Total aerobic bacteria were counted on nutrient agar, faecal coliforms on VRB agar and faecal streptococcus counts were determined on KF Streptococcus agar (Oxoid) supplemented with TTC (Sigma).

Statistical analysis was performed with SPSS 12.0. Analysis of variance was used when data were distributed in a normal function (Shapiro-Wilk test, significance > 0,200) and homogeneity of variances assumption was accepted regarding to Levene's test (significance < 0,050). When differences were found, Tukey test was conducted to compare the three seasons. In the case that distribution was not normal and variances were heterogeneous, Welch's robust test of equality of means was applied (Reed and Stark, 1988). The number of positive manures or farms was compared by non parametric tests (chi-squared tests).

EXPERIMENTAL DATA AND RESULTS

The results related to physicochemical composition are expressed as: mean ± standard error. The upscaled facilities produced more diluted organic wastes (percentage of dry matter: 11.75 ± 1.05) than traditional ones (14.48 ± 1.46), with significance < 0.050. More saline manures were founded in upscaled farms (4.280 ± 1.489 *vs* 1.184 ± 0,148) with significance < 0.100.

Seasonal changes on composition of manures were also observed, as can be seen in Table 1. The pH and conductivity were different in autumn compared with summer or spring values, in the second cluster studied. On the other hand, in the third cluster, pH, conductivity and ammonia concentration showed differences in summer, when compared with spring or autumn.

Table 1. Physicochemical composition of manures, by season and cluster. Mean ± standard error. ^{a,b}: Values with different superscript letters are significantly different. ***: significance < 0.050 and *: < 0.200

PARAMETER	SEASON	CLUSTER 2	CLUSTER 3
pH	Spring	8.06 ± 0.27^{a}	7.95 ± 0.24^{a}
	Summer	7.88 ± 0.16^{a}	$8.68 \pm 0.08^{b\ ***}$
	Autumn	$8.22 \pm 0.13^{a,b\ *}$	8.23 ± 0.13^{a}
Conductivity	Spring	5.596 ± 2.767^{a}	1.212 ± 0.105^{a}
	Summer	6.247 ± 3.394^{a}	$0.651 \pm 0.067^{b\ ***}$
$N\text{-}NH_3$ (%)	Autumn	$0.995 \pm 0.100^{b\ *}$	1.285 ± 0.261^{a}
	Spring	0.14 ± 0.03^{a}	0.14 ± 0.02^{a}
	Summer	0.14 ± 0.02^{a}	$0.09 \pm 0.03^{b\ *}$
	Autumn	0.13 ± 0.01^{a}	$0.13 \pm 0.03^{a,b}$

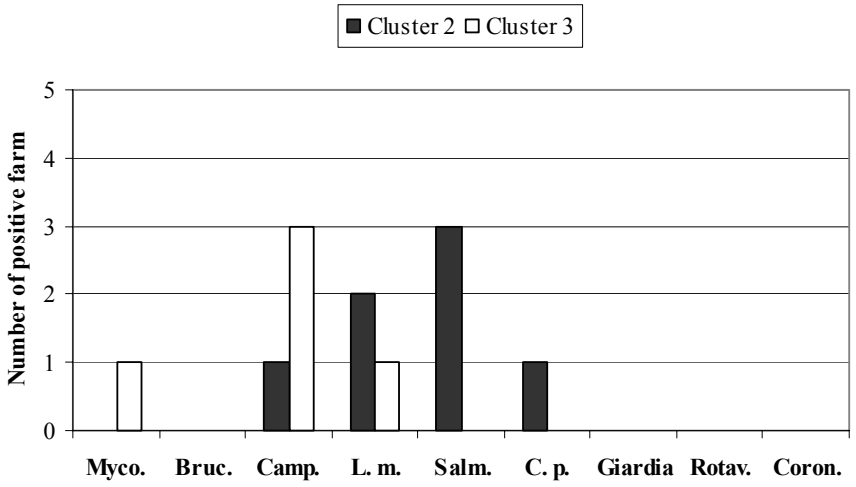
Regarding to bacterial indicators which are linked to faecal contamination, corresponding data are indicated in Table 2. When upscaled farms' manures were tested, the means of total aerobic bacteria and faecal coliforms' counts were significantly different in autumn *vs* summer values. On the other hand, traditional farms' manures presented seasonal differences in the mean of faecal streptococcus counts when comparing summer with spring or autumn.

When positive manures were estimated for each season, results revealed spring (5 positive manures out of 10) and summer (5 out of 10) as the seasons in which the higher number of manures presented zoonotic agents, followed by autumn (3 out of 10). Although in autumn, second cluster included two positive farms compared with only one positive farm in third cluster, neither the number of pathogens nor the number of positive manures or farms gave enough frequency in each cell of Chi tests to ascertain the influence of season alone or season combined with cluster. Nevertheless, statistical analysis showed that the upscaled farms had similar incidence of positive manures (7 positive manures out of 15) than the traditional ones (6 out of 15), when the data of all the seasons were grouped. The corresponding Chi test obtained a significance of 0,713, giving that none of the cells had less than 5 observations and that the minimum expected frequency was 6,50.

Table 2. Logarithm of bacterial counts, by season and cluster. Mean ± standard error. [a,b]: Values with different superscript letters are significantly different. [***]: significance < 0.050, [**]: < 0.100 and [*]: < 0.200

PARAMETER	SEASON	CLUSTER 2	CLUSTER 3
Total aerobic bacteria	Spring	7.48 ± 0.25[a]	7.56 ± 0.30[a]
	Summer	7.39 ± 0.18[a]	7.59 ± 0.19[a]
	Autumn	7.93 ± 0.13[a, b ***]	7.58 ± 0.17[a]
Faecal coliforms	Spring	4.60 ± 0.52[a]	5.68 ± 0.26[a]
	Summer	4.36 ± 0.49[a]	4.46 ± 0.98[a]
	Autumn	5.32 ± 0.32[a, b *]	5.74 ± 0.07[a]
Faecal streptococci	Spring	5.46 ± 0.56[a]	6.05 ± 0.25[a]
	Summer	4.62 ± 0.87[a]	4.18 ± 0.76[b] **
	Autumn	5.54 ± 0.37[a]	6.15 ± 0.20[a]

Graph 1 represents the frequency for each of the zoonotic agents investigated in the two clusters. It is necessary to remark that the zoonotic agent present in each season and farm type was different. So, upscaled farms had higher frequency of these pathogens: *L. monocytogenes*, *Salmonella* spp. and *C. parvum* while the zoonotic agents that prevailed in traditional farms were *Campylobacter* spp. and mycobacteria. Moreover, the presence of *L. monocytogenes* in upscaled farms' manures was not detected in summer, while it did not appear in traditional manures in autumn. *Campylobacter* spp. was present in the summer manures of second cluster, but in summer and autumn manures of third cluster. Manures positive for *Salmonella* spp. were sampled in spring, summer and autumn.



Graphic 1. Number of positive farms for each of the zoonotic agents, out of five farms by cluster

DISCUSSION

Regarding to manures' composition in the two clusters studied, the traditional farms tended to produce manure with higher solid content and lower saline content. So, the quality of traditional manures for fertilizing is better than the one of second cluster manures and the spreading of their manures could be presented as more environmentally friendly. Experimental data showed autumn as the season in which upscaled farms produced less saline manure and with higher pH, while traditional manures in summer were less saline, had lower ammonia content and higher pH. So, the use of these wastes as fertilizer could be encouraged in autumn for the second cluster farms and considered in early summer for the third cluster farms.

Moreover, second cluster manures presented in autumn higher number of total aerobic bacteria and of faecal coliforms, while faecal streptococcus counts remained constant. So, it would be more useful to choose total aerobic bacteria or faecal coliforms as bacterial indicators of faecal contamination. In third cluster manures, on the contrary, the outstanding indicators are faecal streptococci, which counts are higher in summer.

The pathogens selected in this work affect not only to dairy cows but to humans, and are listed as zoonotic agents to be monitored (Directive 2003/99/CE). The pathogens isolated in upscaled farms could be related to in-house stocking density, as Hutchison *et al.* (2005) found lower levels of *Cryptosporidium* oocysts in pig wastes when stocked at densities lower than 0.4 animal m^{-2}. Pathogens identified in both clusters are hygienic conditions dependant, for instance Nightingale *et al.* (2005) indicated that maintaining adequate animal hygiene prevented against listeriosis in cattle farms. The presence of *Salmonella* spp. in the upscaled farms' manures is also underlined by the fact that it was the only pathogen detected in the three seasons studied. In this way, Venglovský *et al.* (1998) related literless technology and liquid manures to *salmonellae* ideal conditions for survival.

CONCLUSIONS, SCIENTIFIC AND/OR PRACTICAL IMPLICATIONS OF THE WORK

These evidences show that changes in dairy facilities and manure management must be carefully tested so that health risk is not compromised. The higher numbers of cows, the dilution of manure or the reduced time of storage associated with upscaled farms could be related to the isolated pathogens. Anyhow, traditional management influences the type of pathogens being present, as well as it produces a fertilizer of higher quality which is more environmentally friendly, including smaller energy consumes. The type of pathogens isolated in upscaled farms could be the ones related to big herds and liquid slurry and the ones identified in both clusters could be hygienic conditions dependant. The high incidence of *Salmonella* spp. in second cluster and of *Campylobacter* spp. in third cluster should be further investigated, so that proper manure management or reducing of risk factors is achieved and the dissemination of these relevant pathogens is avoided. The detection of *Salmonella* spp. from spring to autumn in upscaled farms should be corrected and no incidence of *L. monocytogenes* in autumn should be researched in traditional farms.

ACKNOWLEDGEMENTS

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COMPARISON OF AEROBIC AND ANAEROBIC WAYS OF TREATMENT OF PIG SLURRY SOLIDS

Venglovský, J., Sasáková, N., Vargová, M., Ondrašovičová, O., Ondrašovič, M. and Martinez, J.

University of Veterinary Medicine, Komenského 73, 041 81 Košice, The Slovak Republic
E-mail: jan@venglovsky.com

SUMMARY

Temperature development was observed on a laboratory scale in the solid fraction of pig slurry amended with 1 and 2% powder zeolite during storage under anaerobic and aerobic conditions. The highest temperatures reached in the controls were 31.5^{o} C and 36.9^{o}C (anaerobic and aerobic, resp.). In zeolite-amended substrates the temperatures were 29.8^{o} C (anaerobic) and 30.0^{o} C (aerobic). The plate counts of faecal coliforms decreased more in zeolite-amended substrates compared to the control. None of the substrates were considered hygienically safe after the storage.

Keywords: pig slurry, zeolite, aerobic and anaerobic storage, microbial plate counts

INTRODUCTION

Excrements of animals from systems with bedding produce little risk as their biothermic processing can be ensured easily and the final product is hygienically safe and suitable for application to agricultural soil. However, treatment and disposal of slurry presents frequently serious problems intensified by large volumes of this form of animal excrements and their potential environmental consequences. Many pathogenic micro-organisms can accumulate in the slurry and remain vital for different periods depending on many factors. One of the important factors is the treatment of slurry. The frequently used aerobic biological treatment of slurry with activated sludge starts with separation of slurry to the liquid and solid fractions within the primary treatment (Dubinský *et al.,* 2000).

The solid fraction of pigs slurry obtained by separation on vibrating sieves is frequently treated only by simple storing in field dung pits without adding any bulk materials or turning. Composition of pig slurry solids with regard to dry mater content and C/N ratio does not support thermophilic processes and because of that higher temperatures needed for sanitation of this substrate cannot be reached. Decomposition processes in stored, non-aerated pig slurry solids are mostly anaerobic which results in longer time needed for decomposition of complex organic substances to make them suitable for manuring and potential risk of survival of some pathogenic micro-organisms. Many bacteria and helminth eggs can survive under anaerobic conditions for relatively long time Novák *et al.,* 1998, Juriš *et al.,* 2000).

MATERIAL AND METHODS

The solid fraction of pig slurry (21.5% DM) was amended with powder zeolite at a ratio of 1% and 2% by weight (substrates 2 and 3). The substrates were poured loosely to plastic bags, 50 kg substrate per bag, height of the substrate approx. 65 cm (1% zeolite – substrates 2 and 5; 2% zeolite – substrates 3 and 6). Unamended pig slurry solids were used as a control (substrates 1 and 4). All bags were stored in enclosed room and openings were made at the bottom for draining the liquid. Bags 1–3 were closed at the top and were not mixed during the storage to simulate anaerobic conditions. Bags 4–5 remained opened and their content was mixed in specified intervals to introduce some air. Samples were taken and analysed after 6 and 12 weeks of storage.

Chemical examination included determination of DM, ash, N_t and P_t. Microbiological examination consisted of determination of plate counts of psychrophilic, mesophilic, coliform and faecal coliform bacteria.

RESULTS AND DISCUSSION

The development of temperature in the core of substrates is shown in Fig.1 and Fig. 2.

During the first 6 weeks of storage, the highest temperature was recorded in the control substrates 1 and 4 (31.5 and 36.9°C) while in those with zeolites they did not exceed 29.8°C and 30.0°C (anaerobic and aerobic, resp.). During the second half of the experiment, the highest temperature (33.1°C) was recorded in the aerated substrate with 2% zeolite and in anaerobic substrate with 1% zeolite (29.7°C). External temperature varied in the range 18–23°C.

Our experiment with pig slurry storage under laboratory conditions showed that thermophilic range of temperatures necessary for sanitation of this material was not reached. A positive influence of zeolite was observed only in the later period of storage (after 6 weeks). The content of moisture at the beginning of the experiment was in the range 76.5–78.5% which, together with insufficient aeration, can be considered the main reason for not reaching the thermophilic phase (Tiquia *et al*., 1998).

The results obtained showed that the release of individual forms of nitrogen, particularly of ammonia nitrogen, into water extract was slower and more uniform in the presence of zeolite. Concentrations of $N\text{-}NO_3$ indicated that more favourable conditions for nitrification developed in the substrate with 1% zeolite. Gradual release of nutrients into water extracts from the substrates with zeolite was indicated also by values of electrolytic conductivity. After 3 weeks of storage we observed an increase in P-fixation capacity in the substrate with 2% zeolite, more pronounced in the aerated substrates (Fig. 3 and 4), which is in agreement with the observations of Sakadevan and Bavor (1998). In dependence on respective conditions of storage (anaerobic, aeration) we observed differences in decomposition processes, reflected in pH, dry mater content and phosphorus, in favour the aerated substrates similar as those observed by Day and Shaw (2000).

Considerable quantity of liquid retained in the substrate is released in the first stage of storage of pig slurry solids. This liquid may penetrate into soil and eventually into ground water if capture of dung water is not ensured. Our study showed that addition of zeolite decreased considerably the volume of dung water released during the first 48 h of storage. Also the concentration of CHSK, N_t, P_t, $N\text{-}NH_3$, $N\text{-}NO_3$ and EC in released dung water was lower. The influence of zeolite in this direction was dose dependent (Tab. 1).

Plate counts of psychrophilic, mesophilic and coliform bacteria corresponded more or less to the course of temperature in the respective substrates. (Fig. 5 and 6). Differences between

substrates with zeolite and the control were insignificant throughout the experiment. However, with regard to faecal coliform bacteria, which are considered indicators of sanitation, we observed their decreased survival in the substrates with zeolite compared to control substrates in the last stage of storage. Their lowest plate counts were found after 3 and 6 weeks of storage in the substrate with zeolite which was aerated by turning.

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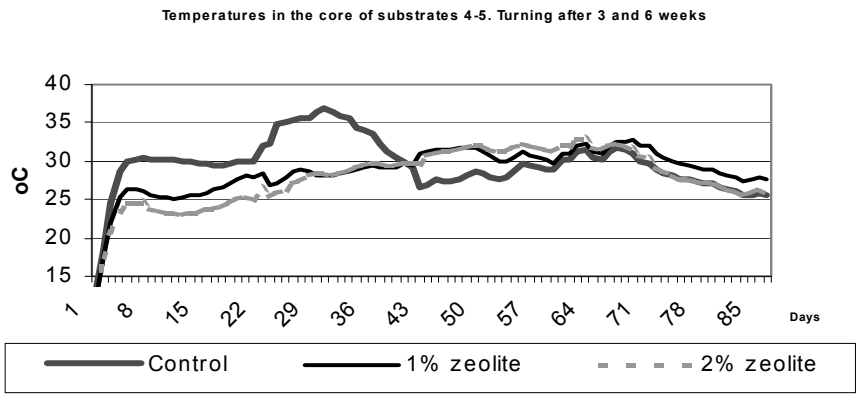

Figure 1. Temperatures in the core of substrates 1–3. anaerobic condition

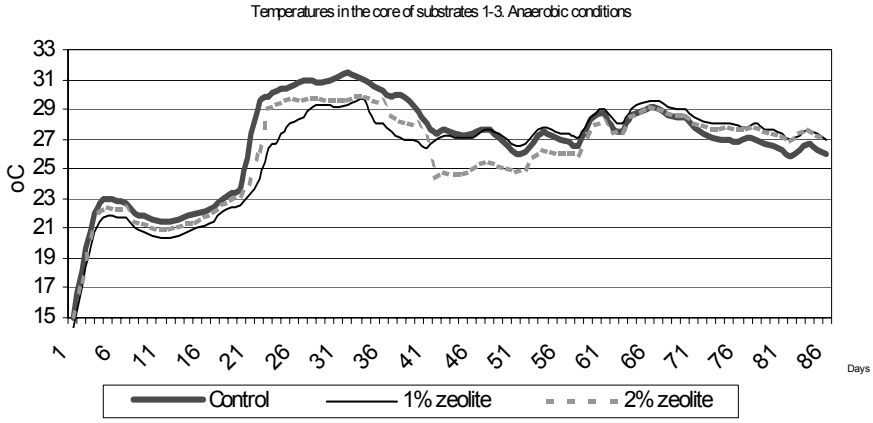

Figure 2. Temperatures in the core of substrates. Turning after 3 and 6 weeks aerobic conditions

Table 1. Concentration of nutrients in the liquid released from stored substrates and their decrease in comparison with the control in %

	Control substrates (1, 4)	1% zeolite – substrates (2, 5)	2% zeolite – substrates (3, 6)
N_t [g]	22,504	15,983 (28,98%)	13,538 (39,84%)
N-NH_3 [g]	16,612	12,074 (27,32%)	10,57 (36,37%)
N-NO_3 [g]	4,637	3,151 (32,0%)	2,59 (44,11%)
P_t [g]	0,898	0,707 (21,2%)	0,594 (33,85%)
COD[g]	169,361	122,942 (27,4%)	106,785 (36,95%)

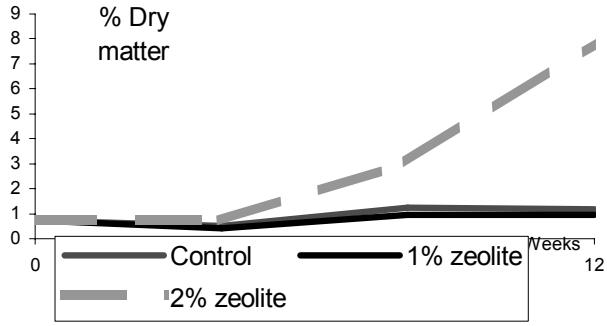

Figure 3. P_t – anaerobic conditions

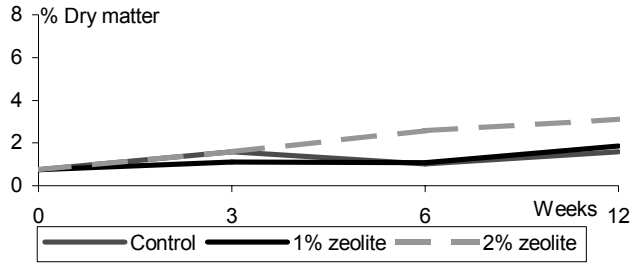

Figure 4. P_t – aerobic conditions

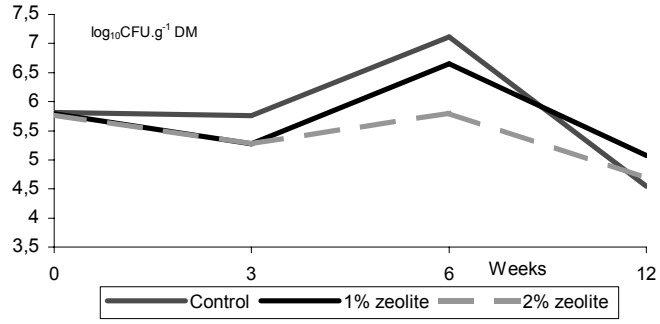

Figure 5. Coliform bacteria – anaerobic conditions

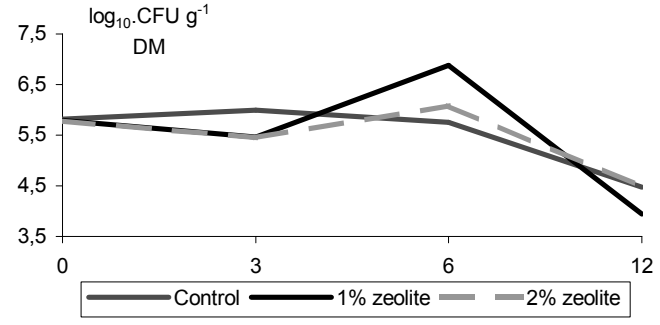

Figure 6. Coliform bacteria – aerobic conditions

EFFECT OF ANAEROBIC STORAGE AND AEROBIC DIGESTION ON MICRO-ORGANISMS IN PIG MANURE: CULTURAL AND MOLECULAR APPROACHES

Pourcher, A.-M.[1,2], Marti, R.[1], Thorigné, A.[1], Jégou, B.[1] and Dabert, P.[1]

[1] Unité de Recherche Gestion environnementale et traitement biologique des déchets CEMAGREF-17, avenue de Cucillé – CS 64427, 35044 Rennes cedex, France;
[2] Laboratoire Paysages et Biodiversité-PPF DS 10, -2 boulevard Lavoisier, 49045 Angers cedex 01, France

SUMMARY

The impact of manure treatment was studied on bacterial populations using cultural methods and 16S rRNA targeted PCR Single-Strand-Conformation-Polymorphism analysis (SSCP). Aerobic treatment followed by anaerobic storage resulted in a reduction of between one to two logarithmic units of the numbers of *E coli* and enterococci, but this was not sufficient to eliminate *Salmonella* and *Listeria monocytogenes*. The dominant microbial community of the raw manures remained very stable with the persistence of four species whereas SSCP profiles of treated manures showed a greater diversity of bacterial population. Two species found in raw manure, *Bifidobacterium thermacidophilum subsp. porcinum* and *Lactobacillus sobrius*, could be proposed as manure indicators.

Keywords: manure, treatment indicators, pathogen, survival, SSCP, 16S rRNA

INTRODUCTION

Effluents from piggeries, that may contain viruses, parasites and pathogenic bacteria, can present a sanitary risk during its subsequent spreading on agricultural land. It has been observed that spreading has resulted in an increase in number of pathogenic microorganisms in soil (Gessel *et al.*, 2004). The health risk increases when manure is spread on soil where certain crops (e.g. salads, fruit and some vegetables) that are not intended to be cooked are grown (Nicholson *et al.*, 2005). In response to the recent strengthening of European regulation concerning the recycling of animal by-products (regulation n° 1774/2002), it is important to study the effectiveness of manure treatments which include aerobic processes developed primarily for biological nitrogen removal.

The behaviour of the microorganisms in manure is generally studied using either cultural or molecular methods. The cultural approach is adapted to the detection of indicator bacteria (*E. coli*, enterococci and *Clostridium perfringens*) and specific pathogenic bacteria. It enables numeration of low levels of pathogens. Nevertheless, the absence of specific media and the existence of viable but non cultivable forms introduce a bias on the number detected. Thus, among the 10^9 to 10^{10} bacteria /mL enumerated in manure using direct microscopic counting, only 10 to 20% can be cultured (Cotta *et al.*, 2003). Furthermore, the inventory of the faecal flora of 24 pigs revealed the presence of 375 phylotypes (molecular equivalent of a species) for which only 17% were close to

known species (Leser *et al.*, 2002). The molecular approach may thus appear more relevant for the detection of faecal microorganisms even if, it cannot yet target subdominant microbial groups. As expected, no pathogenic bacteria or classical bacterial indicator have been detected during the molecular inventories of faecal matter and manure (Leser *et al*., 2002, Snell-Castro *et al.*, 2005).

The aim of this study was to compare the impact of the simple anaerobic storage of manure with a more complex aerobic/anoxic treatment on the bacteria of sanitary interest and on the manure dominant microbial community. The originality of the study consisted in the use of both cultural and molecular methods.

MATERIAL AND METHODS

Sampling was carried out between March and July 2006 at 17 piggeries located in Brittany (France). Samples were taken from 27 anaerobic storage tanks : 17 from the raw manure storage tanks (primary tank) and 10 from the treated manure storage tanks after aerobic digestion (secondary tank). Before sampling, manure that had been stored in the tanks between 3 weeks and 6 months were homogenised by mixing. Microbial analysis was performed by (i) cultural methods and (ii) PCR amplification of the 16S ribosomal RNA bacterial genes followed by Single-Strand Conformation Polymorphism analysis (SSCP) of the PCR products.

Cultural methods

E. coli was enumerated using 3M Petrifilm *E. coli* (incubated 24 hours at 44°C). Detection of *E. coli* was based on enumeration of blue colonies according to the manufacturers' directions. Enterococci were enumerated as black colonies on Bile Esculine Azide agar (4 h, 44°C) resulting from a transfer of the red colonies obtained on a first plating onto Slanetz-Barkley agar (48 h, 37°C). Spores of *Clostridium perfringens* were enumerated according to the Most Probable Number (MPN) method previously described by Pourcher *et al.* (2006). *Salmonella* was enumerated according to the MPN method described by AFNOR (Anonymous, 2004). The presence of *Listeria monocytogenes* was detected in ten grams of manure using an Oxoid Novel Enrichment Broth-Listeria (ONE Broth) (24h, 30°C) followed by plating onto a chromogenic Agar Listeria according to Ottaviani and Agosti (ALOA). Typical colonies on ALOA agar were subjected to PCR for lysteriolysin O genes (*hlyA*) detection using the primers previously described by Bohnert *et al.* (1992).

Molecular analysis

Manure samples were centrifuged for 10 min at 17,500 g. About 0.25 g of each pellet was transferred into a microtube and immediately stored at – 20°C. DNA extractions were performed on one pellet using the QIAamp DNA mini stool kit (QIAGEN). The manure microbial communities were analyzed by PCR amplification of the V3 region of microbial 16S rRNA genes using primers targeting the total bacteria or specific microbial groups as described by Peu *et al.* (2006) and Matsuki *et al*. (2004). Four microbial groups were targeted: *Bacillus-Streptococcus-Lactobacillus* (BSL), *Eubacterium-Clostridium* (EC), *Bacteroides-Prevotella* (BP) and *Bifidobacterium*. Amplification was performed with a MJ Mini thermocycler (Bio-rad). The resulting PCR products were then separated by SSCP capillary electrophoresis with an ABI 310 genetic analyzer (Applied Biosystems) as described by Delbes *et al.* (2000). The dominant populations observed after SSCP electrophoresis were identified by the cloning and sequencing of there corresponding PCR product according to the methodology previously described by Peu *et*

al. (2006). DNA sequences were identified by comparison with their closest relatives available in databases using BLAST from the National Centre for Biotechnology Information and the Ribosomal Database Project.

RESULTS

Faecal indicators dynamic in pig manure

Overall, for the 17 piggeries, indicator counts in raw manure varied from 6×10^3 to 1×10^8 for *E. coli*, from 6×10^4 to 7×10^6 for enterococci and from 2×10^3 to 1×10^6 for *C. perfringens* per g of wet weight. The variation observed (about 2 logarithmic units) for *E. coli* and enterococci are in the same order of magnitude than those reported by Hill and Sobsey (2003) and Vanotti *et al.* (2005) for liquid manures. *Salmonella* and *L. monocytogenes* were detected in 60 and 30% of the 17 raw manure samples respectively. The proportion of *Salmonella* is in agreement with those reported by Chinivasagam *et al.* (2004) and Watabe *et al.* (2003) who isolated *Salmonella* respectively in 31% and 71.4% of the analyzed manures. The difference of proportion and levels of *Salmonella* in manures are affected by numerous factors such as geographic location, size of the breeding, age of livestock and dietary changes. The presence of *L. monocytogenes* is probably due to its ubiquitous character as it was suggested by Garrec *et al.* (2003) who regularly detected this bacterium in sludge of waste water treatment plants. The 10 treatment systems studied showed a reduction of *E. coli*, enterococci and *Salmonella* (Table 1), with average reductions of 3.1 $\log_{10}$ for *E. coli*, 1.4 $\log_{10}$ for enterococci, 1.2 $\log_{10}$ for *Salmonella* but only 0.2 $\log_{10}$ for *C. perfringens*. The absence of reduction of *C. perfringens* spores confirms the large resistance of this bacterium to the treatment, as it was previously observed by Pourcher *et al.* (2005) during the composting of municipal sludge.

Table 1. Concentrations of bacteria (per gram of wet weight) and occurrence of *L. monocytogenes* in raw manures and the treatment by-products from 17 piggeries

manure treatment	type of manure		*E. coli*	*Enterococci*	*C. perfringens*	*Salmonella*	*L. monocytogenes*
anaerobic storage[a]	raw manure	mean	**$3.5\ 10^5$**	**$8.3\ 10^5$**	**$3.5\ 10^4$**	**41**	**0%[e]**
	(7)[c,]	(SD)[d]	($4.1\ 10^5$)	($1.4\ 10^6$)	($2.9\ 10^4$)	(99)	
anaerobic storage[b]	raw manure	mean	**$1.1\ 10^7$**	**$1.9\ 10^6$**	**$2.0\ 10^5$**	**3.8**	**50%**
	(10)	(SD)	($3.1\ 10^7$)	($2.2\ 10^6$)	($3.7\ 10^5$)	(7.4)	
aerobic digestion followed by anaerobic storage[b]	treated manure (10)	mean (SD)	**$9.7\ 10^3$** ($4.1\ 10^3$)	**$7.1\ 10^4$** ($5.9\ 10^4$)	**$1.4\ 10^5$** ($2.4\ 10^5$)	**0.2** (0.5)	**20%**

[a] piggeries without N removal treatment; [b] piggeries with N removal treatment; [c] number of samples; [d] standard deviation;
[e] frequency of detection of *Listeria monocytogenes* (%)

The succession of aerobic digestion and anaerobic sludge storage clearly affected the survival of vegetative bacterial forms. However, faecal indicators and pathogen bacteria were present in treated manure and persisted up to the time of spreading onto the fields.

Bacterial 16S rRNA gene dynamic in manure

The composition of the raw manures microbial communities was determined by 16S rRNA gene-targeted PCR amplification and subsequent SSCP electrophoresis of the PCR products (Figure 1). This technique allows the representation of a microbial community as a profile of peaks where each dominant peak is representative of a PCR product and by implication, a bacterial species. The SSCP profiles obtained for the 17 raw manures were relatively homogeneous regardless of the time of manure storage (ranging from 3 weeks to 6 months), suggesting a weak impact of the anaerobic storage on the microbial community. The profiles contained about 32 distinguishable peaks that emerged from a background of subdominant bacterial diversity (Figure 1a). As observed previously by Peu *et al.* (2006) during anaerobic manure storage, the profiles could be subdivided in 3 groups of peaks corresponding to the *Clostridiaceae*, *Bacteroidetes* and BSL microbial groups. By contrast, the SSCP Profiles obtained for the 10 treated manures differed strongly from those of raw manures by a greater apparent diversity of peaks (Figure 1b). These changes of profiles reveal an important evolution of the corresponding microbial communities and thus a strong impact of the aerobic treatment on the raw manure as previously observed by Leung and Topp (2001). The harsh conditions of aerobic treatment probably inhibited some of the strict anaerobic microbial groups of raw manure and supported the growth of other environmental species. These important changes in the dominant microbial groups correspond also to the decrease of the subdominant groups to which belong indicator bacteria.

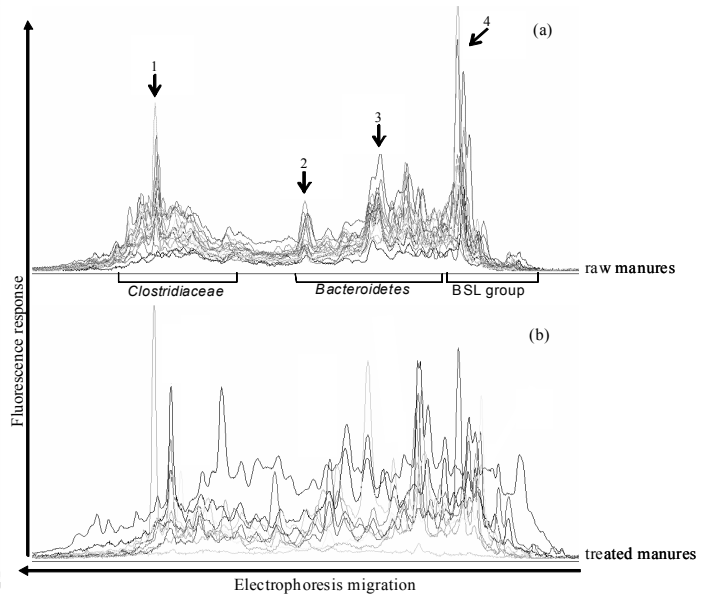

Figure 1. Comparison of bacterial 16S rRNA gene-targeted PCR – SSCP profiles for 17 raw manures sampled in primary storage tanks (a) and for 10 treated manures sampled in secondary storage tanks (b). SSCP electrophoresis was performed from right to left. The horizontal and vertical axes indicate time (number of scans) and the detection of fluorescently labelled PCR products, respectively. Arrows indicate dominant peaks present in a majority of raw manures.

The search for new bacterial indicators of pig manure

Interestingly, four of the dominant peaks of raw manures (noted as 1 to 4 on figure 1a) were present in the majority of the samples analysed. They were investigated to see if they could be used as microbiological markers of pig manure. They were all related to uncultured bacteria. Peak 1 was closely related to *Clostridium* (accession number DQ309375) with a 93% sequence similarity. Peaks 2, 3 and 4 were related to *Bacteroidales* with 92–93% sequence similarity (accession numbers AB240481, AB240481 and AB175368, respectively). As these 4 species were not specific to pig manure, the same strategy was carried out again but targeting more specific bacterial subgroups (EC, BP, BSL groups and *Bifidobacterium*). Two SSCP peaks were remarkably present in several raw manures. They were assigned to *Bifidobacterium thermacidophilum subsp. porcinum* (accession number AY148470, sequence similarity of 98%) and *Lactobacillus sobrius* (accession number AY700063, sequence similarity of 99%), two species previously identified in intestinal tract of both pig and piglet (Zhu and Dong, 2003; Konstantinov *et al.*, 2006). However, comparison with other livestock effluents (poultry and cattle) is in progress to confirm their specificity.

CONCLUSION

The value of this study lies in the large number of piggeries included which enables the determination of the general characteristics of the bacterial community of raw and treated manures. The results underline the existence of a potential risk of spreading *Salmonella* which were detected in 60% of the 17 raw manures and in 20% of the 10 treated manures analysed. The N removal treatment results in a decrease in *E coli* and enterococci concentrations, but is not however sufficient to completely eliminate the pathogenic bacteria and it has no effect on the spores of *C perfringens*. The molecular analyses highlighted (i) the strong impact of aerobic treatment on the raw manure microbial community and (ii) the presence of specific populations in the raw manures belonging to *Bifidobacterium* and *Lactobacillus* groups that could be proposed as new indicators of the effectiveness of treatment.

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DETERMINATION OF ANTIBIOTIC RESIDUES IN LEACHATE OF CONVENTIONAL AND ORGANIC DAIRY FARMS

Kemper, N.[1], Färber, H.[2], Skutlarek, D.[2] and Krieter, J.[1]

[1] Institute for Animal Breeding and Husbandry CAU Kiel, Olshausenstraße 40, 24098, Kiel, Germany, nkemper@tierzucht.uni-kiel.de; [2] Institute for Hygiene and Public Health Bonn

SUMMARY

The scientific interest in antibiotic substances in the environment has increased. The assumed quantity of antibiotics excreted by animal husbandry reaches thousands of tons per year. The environment is contaminated by these compounds on different pathways. To verify if animal husbandry can be a source of antibiotic residues showing up in the environment, manure and leachate samples from dairy farms were analysed. Neither in liquid manure nor in leachate were analysed antibiotics detected. As the main concern regarding the use of antibiotics is the development of resistant bacteria strains, further studies are implicitly required.

Keywords: agriculture, antibiotics, leachate, manure, residues

OBJECTIVES

More than 50 pharmaceutical compounds, or their metabolites, respectively, have been isolated from ground- and even drinking water (MÜCKTER, 2006). Even though the analysed concentrations are on a low level in areas next to drinking water resources, a possible impact on human and animal health can not be excluded. Therefore, and due to the recent achievements in the field of modern analytical techniques, the scientific interest in antimicrobially active compounds in the environment has increased during the last decade. There are three risks deriving from immoderate appliance of antibiotics resulting in environmental contamination with original substances or derivatives: the indirect impact on human and animal health via resistant micro-organisms, the direct organic damage and the influences on the biotic environment are a matter of concern.

In human as well as in veterinary medicine, antibiotics are used to treat and prevent disease. However, they are not completely eliminated in the organism, as they are bioactive substances, acting highly effectively at low doses and excreted after a short time of residence. Antibiotics are optimised with regard to their pharmacokinetics in the organisms: organic accumulation is, as in other pharmaceutics, objectionable and thus, they are excreted as parent compounds or metabolites (KÜMMERER et al., 2000; THIELE-BRUHN, 2003). Excretion rates are dependant on the substance, the mode of application, the excreting species and time after administration, but it has been shown that rates vary between 40% to 90% for tetracyclines and sulphonamides (BERGER et al., 1986; HALLER et al., 2001; HALLING-SØRENSEN, 2001). Administered medicaments, their metabolites or degradation products reach the aquatic environment by the application of manure or slurry to areas used agriculturally, or by pasture-reared animals excreting directly on the land, followed by surface run-off, driftage or leaching in deeper layers of earth (Figure 1).

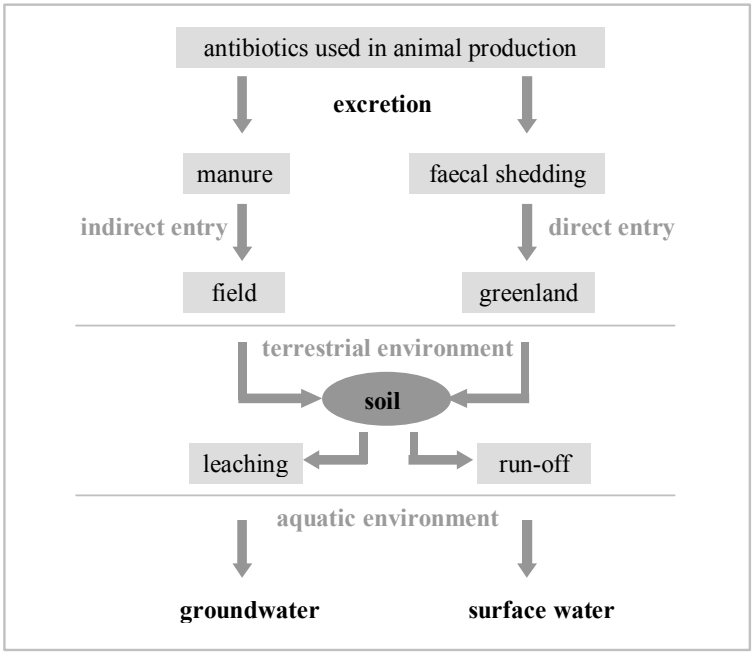

Figure 1. Exposure pathways of veterinary antibiotics in the environment

Concentration limits of antibiotics in the environment are not regulated, even though the growing concern has been taken into account with the prescription of environmental risk assessment of veterinary pharmaceuticals in the U.S and Europe (EMEA, 1997; THIELE-BRUHN, 2003). Risk assessment is realised by the calculation of predicted environmental concentrations (PEC) and comparison with predicted, biological non-effective concentrations (PNEC) (KÜMMERER, 2001a).

In Germany as well as in other countries, regulations concerning drinking water quality demand to hold down the amount of chemical contaminants polluting or altering the quality of drinking water as low as possible. To implement these regulations, the knowledge of the distributions of antibiotics in the environment is essential. The objective of this study was to verify, if animal husbandry can be a source of antibiotic residues showing up in the aquatic environment in Northern Germany. Furthermore, the occurrence of these residues and potential differences in conventional and organic farms were evaluated.

METHODS

Liquid manure samples were collected during two seasons of leaching (October until February) in 2004/05 and 2005/06 every four weeks on two conventional and two organic dairy farms in Schleswig-Holstein, Germany. Manure was stirred mechanically in the slurry tank before sampling to guarantee a specimen as homogeneous as possible. All samples were filled into brown glass bottles and placed in the refrigerator (4°C) until further processing (<4 days).

At the same time, leachate samples were taken with help of suction cups on two fields per farm, one of grassland and one of maize. During the test period, the sampling interval of four weeks could not be maintained totally due to weather conditions, as a minimum of two litres of leachate is needed for analysis. In 2004/05, a total of eight samples, containing five manure samples, was analysed. These examinations served as preliminary tests to establish the analysis conditions for manure samples and to adjust the sensitive laboratory methods. In 2005/06, 34 samples were examined, consisting of ten manure samples, eleven leachate samples from grassland and 13 leachate samples from maize fields.

Chemical analysis of antibiotics from different matrices, especially manure, is complicated by the need for extraction. In this study, sample clean-up was performed by a 1:50 dilution with following centrifugation and via solid phase extraction. All samples were analysed with high-performance liquid chromatography in combination with tandem mass-spectrometry (HPLC-MS/MS). High recovery rates of about 70% for oxytetracycline, chlortetracycline, and tylosin enable this method for the analysis of antibiotics in difficult matrices like manure even in the low microgram per kilogram range. Samples were analysed for more than 20 substances listed in table 1. Additionally, to discriminate residues' origins, detailed information of the applied antibiotics on these farms were recorded accurately with questionnaires.

Table 1.

Antibiotics	Limit of quantification (LOQ) in ng/l	Antibiotics	Limit of quantification (LOQ) in ng/l
Amoxicillin	10	Dehydrato-Erythromycin	5
Ampicillin	10		
Benzylpenicillin	10	Roxithromycin	5
Cloxacillin	10	Spiramycin	5
Dicloxacillin	10	Tylosin	5
Flucloxacillin	10	Trimethoprim	5
Methicillin	10	Sulphadimidine	5
Mezlocillin	10	Sulphamethoxazole	5
Nafcillin	10	Ciprofloxacin	10
Oxacillin	10	Ofloxacin	5
Piperacillin	10	Chlortetracycline	25
Phenoxymethylpenicillin	10	Doxycycline	20
Azithromycin	5	Oxytetracycline	20
Clarithromycin	2	Tetracycline	20
Clindamycin	5	Vancomycin	50

RESULTS

Regarding the application of antibiotics, no differences between conventional and organic farms were assessed neither in the range of applied antibiotics nor in the amount of administered treatments. In general, most treatment took place after mastitis and diarrhoea with ß-lactam-antibiotics and tetracyclines. Out of the list of examined pharmaceuticals, amoxicillin, ampicillin, benzylpenicillin, cloxacillin, sulphadimidine, chlortetracycline, doxycycline, oxytetracycline and tetracycline were administered during the examination period. Furthermore, animals were treated with enrofloxacin and cephalosporines, but these substances could not be analysed by the used methods.

Limits of quantification were not exceeded in any sample, regardless of sample origin. Neither in liquid manure nor in leachate were the analysed antibiotics detected on levels above the limits of quantification. In two manure samples from the sampling period 2004/05, traces of sulphadimidine were found, laying above the limit of detection (2 ng/l), but beneath the limits of quantification (5 ng/l) and therefore not further quantifiable.

CONCLUSIONS

Compared to pig and poultry production, the use of antibiotics in dairy farming is still on a relatively low level, at least at the examined farms. The results can be attributed to the fact that the administered antibiotics either degrade very fast in the environment like ß-lactam-antibiotics or are highly adsorbent to soil like tetracyclines. The structure of ß-lactams such as penicillin, benzylpenicillin or cloxacillin, consisting of the ß-lactam-ring, contribute to the poor stability of this group in the environment: the ring can be opened by ß-lactamase, a widespread enzyme in bacteria, or by chemical hydrolysis. Thus, intact penicillins are usually not found in the environment (MYLLYNIEMI et al., 2000). Neither tetracyclines nor tylosin were detected in any water sample by HAMSCHER et al. (2002). Further confirmation of these findings is supported by LINDSEY et al. (2001) and ZHU et al. (2001). However, these substances have been detected in low levels in U.S. surface water samples (KOLPIN et al., 2002) and in higher levels in overland flow water (KRAPAC et al., 2005). In Northwest Germany, a study was conducted sampling a series from surface waters, detecting a wide range of antibiotics in all samples (CHRISTIAN et al., 2003): sulphonamides, macrolides and lincosamides were analysed frequently, whereas ß-lactams were rarely found. Tetracyclines were not detected because of their strong adsorption to organic matter. The presence of tetracycline-resistant bacterial isolates in lagoons and groundwater underlying two swine production facilities was published by CHEE-SANFORD et al. (2001). MACKIE et al. (2006) detected both tetracycline residues and tetracycline resistance genes in groundwater impacted by swine production facilities. However, antibiotic input by agricultural use is the minor origin of antimicrobials in the aquatic environment. Most of the analysed substances originated from discharge or sewage into rivers, only for a couple of samples could an influence of animal husbandry on the occurrence of antibiotics in surface waters be assumed. The major part of antibiotic input is carried out by human administration via hospital effluents or municipal wastewater, as reviewed by KÜMMERER (2001b).

Even if the occurrence, effects and fate of antibiotics have been put in the perspective of the scientific interest, still little is known about the actual risk to both humans and the environment. Significant gaps still exist in the understanding of the interaction between residues, metabolites and resistance promotion after excretion. But the consequences of increasing resistance in bacteria and the diminishing impact of therapeutic drugs reach far beyond geographic origins of antimicrobial compounds and are therefore of global concern. Without a doubt, a promising approach for proper risk assessment and management is the reduction of the emission of antibiotics into the environment, whether of human or veterinary medical origin. Appropriate use of antimicrobials in livestock production will preserve the long-term efficacy of existing antibiotics, support animal health and welfare and limit the risk factors of transferring antibiotic resistance to animals and humans. Thus, further and deeper studies are implicitly required.

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GREENHOUSE GAS EMISSIONS FROM POULTRY AND PIG PRODUCTION IN SLOVENIA

Dobeic, M., Pintarič Š., Gobec I. and Barlovič N.

Institute for Environmental and Animal Hygiene with Ethology, University of Ljubljana, Veterinary faculty, Gerbičeva 60, 1000 Ljubljana, Slovenia, martin.dobeic@vf.uni.lj.si

SUMMARY

Greenhouse gases carbon dioxide (CO_2), ammonia (NH_3), methane (CH_4) and di-nitrogen oxide (N_2O) were monitored in seven broiler and seven layer stalls, seven pig weaning and fourteen pig fattening stables. Large differences in emissions among stables due to technology, production phases, number, weight and age of animals in poultry and pig production were established. In majority significant ($P<0,05$) climate changes due to variable air streams in stables, which were directly correlated to N_2O concentrations in the air were ascertained. Air stream in fan exhausters was responsible to significant ($P<0,05$) changes in CO_2, NH_3 and N_2O concentrations on exhauster openings. Methane (CH_4) was determinate just in few cases. Enormous N_2O concentrations can be reported in some pig-fattening stables and low infrequent appearance of CH_4 emission.

Keywords: greenhouse gases, emission, climate, poultry, pig production

INTRODUCTION

Animal production is considered to be one of the most important sources of greenhouse gas pollution to the air in Europe. Agriculture's production contribute to 8% greenhouse gases on emissions control level, where animal production share the main source of methane and nitrous oxide (41% each) (1). High ammonia, methane and nitrous oxide emissions into the atmosphere are associated with ecological problems and environmental damage. Besides local impacts emissions from livestock farming contribute to acidification, eutrophication and local disturbance in several regions of Europe. Ammonia can be considered as the key air pollutant as it is emitted in the highest quantities. For this reason most activities on the emissions reduction from animal housing relate to ammonia reduction. (1,2,5) Methane is emitted from human-related activities which beside other include animal husbandry concerning enteric fermentation in livestock, manure and waste management. These activities release significant quantities of methane to the atmosphere. Methane is a greenhouse gas, it affects the ozone layer in the atmosphere and it contributes to global warming or global climatic change (2,3,4). Nitrous oxide is produced as part of the denitrification process of manure, during storage and when manure has been applied to the land (2,3,4). This is the most aggressive greenhouse gas contributing to global warming. Environment burdening policy in Slovenia dealing with national legislation which imposes liability to livestock breeders to report about methane, ammonia and nitrous oxide emissions. Annual permitted emission quotes were described, although measurements can not be executed due diffusion and different sources. Referring to animal age and a detailed inventory of other influences emissions can be estimate. The methodology of livestock breed evaluations as for

emission potentials is not settled in legislation thus evaluation of united assessment greenhouse gases from different housing systems in Slovenia is not possible. In EU legislation maximum and minimum greenhouse emission values can be traced however they vary due to animal housing, meteorological conditions and waste disposal technology what is the reason why they are not useful for real comparison to evaluation in Slovenia. In spite of all that we are further in condition to use only EU values which are not always considered as feasible in practical circumstances.

OBJECTIVES

For a reason to set up fundamentally assessment of greenhouse emissions from livestock production on typical pig and poultry farms in Slovenia a study examining greenhouse emissions was to define existent situation and to establish the meaning of severally individual separate factors over emissions from livestock production in Slovenia. The purpose of this paper was to estimate greenhouse gases and climate in seven broilers, and seven layer stalls, as well as seven pig weaning and fourteen pig fattening stables in Slovenia. To do this, following steps were involved. First, all in- and outdoor measurements we pursued, were monitored in winter (December, January, February) and in early spring (March, April). For this purpose climate (Testo) – temperature (^{0}C), relative humidity (%), air speed (m/s), greenhouse gases (Dräger Multiwarn, Bachrach monitor N_2O) – carbon dioxide (CO_2), ammonia (NH_3), methane (CH_4) and di-nitrogen oxide (N_2O) were monitored. Measurements were performing inside of stables at three points and in three altitudes (10 cm, 80 cm, 200 cm above the floor), meanwhile gas concentrations and air exchange capacity (m^3/h) were determinate inside of fun exhausters. When results were gathered we evaluate the data considering following factors such as farm technology, animal production phases, number, weight and age of animals in time of monitoring. As the fact we look out for hypothesis that emissions from pig and poultry production depend on climate, fodder, sort, category, animal live weight and farming technology, as well as emissions were the highest from pig farms and mutual due to enumerated factors, the objective of study was to establishing recommendations for greenhouse gas emission evaluation from livestock production in Slovenia.

EXPERIMENTAL DATA AND DISCUSSION

Emission data from poultry layer and broiler stables

CO_2, NH_3, CH_4, N_2O concentrations in stables and exhaust air were defined. Each measurement covers average of three broiler stables (age: 14–28 day). Two groups of stables for layer/egg production were defined: Four stables where younger hens (age: 149–232 day) still not lay and three stables where older hens start and resume to lay (age: 279–457 day). In all stables it can be defined that indoor microclimate were strongly influenced ($P<0,05$) to outdoor climate and to stage of age of animal especially according to temperature growth and relative moisture decline. At this time air movement in stables increases progressively.

In average CO_2 concentrations in layer stables represent 0,07 vol% and 0,15 vol% in broiler stables. NH_3 concentrations were higher (15,6 to 95,3 ppm) in stables with younger in the contrast to stables with older hens (14,6 to 92,9 ppm). The same can be concluded for broiler stables where younger categories emitted 5,77 to 14,84 ppm in the contrast to older ones 0,22 to 4,4 ppm.

In average NH_3 concentrations represent 36,61 ppm in all layer and 5,88 ppm in broiler stables. This was significantly ($P<0,05$) linked to lower moisture and higher air movement in layer and broiler stables. Methane CH_4 (5 vol%) was ascertained only 10 centimetres up to the floor in stable for layers when hens were 232 days old, and in stable for broilers when chicken were 18 days old in all three measuring altitudes. N_2O measurements indicate the parallel growth with animal age by concentrations in range from 10 to 98,3 ppm. In layer stables we calculate average N_2O concentrations of 51,85 ppm, in broiler stables 13,70 ppm.

Microclimate parameters and N_2O concentrations in stable air significantly ($P<0,05$) depend to ventilation rates. CO_2, NH_3 and N_2O concentrations in exhaust air significantly ($P<0,05$) depend to ventilation rates as well. Average concentrations in exhaust air emitted from stables for layers were following: CO_2 0,069 vol%, NH_3 37,67 ppm, CH_4 0,00, N_2O 53,61 ppm. Average concentrations from exhaust air of stables for broilers were following: CO_2 0,15 vol%, NH_3 5,88 ppm, CH_4 0,00, N_2O 13,70 ppm.

Calculation considering ventilation rates of total emissions from 10 stables for broiler and layer production has shown that CH_4 emissions can be nearly neglected, meanwhile emissions of N_2O arise with age of animals and achieve nearly 600 ppm in the period when hens reach age of 300 days. After this period emissions of N_2O decline to lower level. With regard to enlarged ventilation rates in ahead of this period this fact is comprehensible, however emissions of N_2O still arise. In broiler stables N_2O emissions did not exceed 50 ppm what can be explained to the time of measuring in early stage of broiler breeding. On the other hand emissions of NH_3 from broiler and layer stables were arising with animal age constantly. Figure 1 show representative results of CO_2, NH_3, CH_4, N_2O emissions from layer and broiler stables considering air streams calculations.

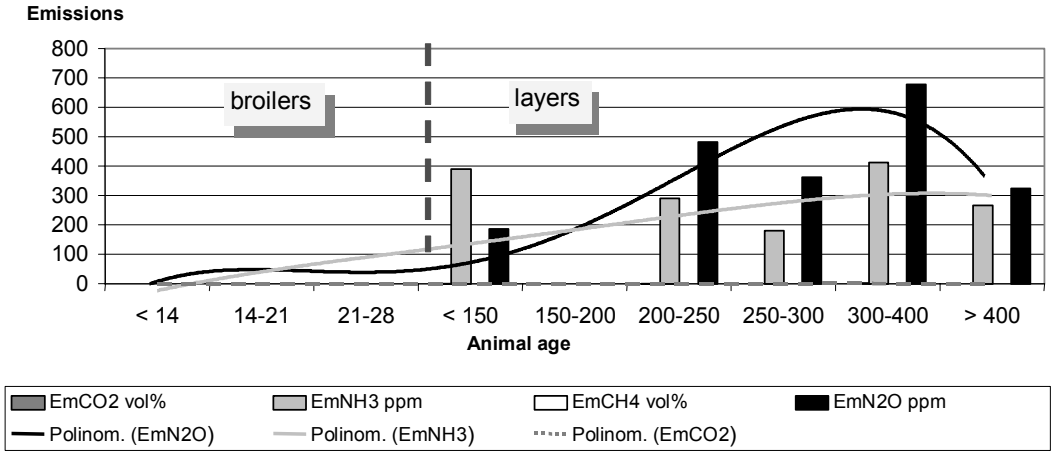

Figure 1. CO_2, NH_3, CH_4, N_2O emissions from layer and broiler stables

Emission data from pig fattening and weaning stables

Two groups for fattening and weaning breeding stables were defined: Fourteen stables where number of fatteners vary between 640 and 900 (body weight: 35 to 105 kg) and seven stables where number of weaners vary between 872 in 962 kg (body weight: 10 in 35 kg). In all stables it

can be defined that indoor microclimate were strongly influenced (P<0,05) to outdoor climate and to stage of age of animal especially according to temperature growth and relative moisture decline.

In average CO_2 concentrations vary between 0,01 and 2,28 vol% in stables settled by fatteners (in average 0,128 vol%), and 0,002 and 0,358 vol% in stables settled by weaners (in average 0,15 vol). NH_3 concentrations were in range 0,49 to 47,0 ppm (in average 9,7 ppm) in fattening stables and 0,06 – 17,71 ppm (in average 7,31 ppm) in weaning stables. CH_4 was detect once on altitude of 2m up to the floor (0,14 vol%) when fatteners had 80 kg and in two cases when weaners had 20 kg in average concentration of 0,064 vol%. When weaners had 15 kg in CH_4 was ascertain in average concentration of 0,58 vol% as well as in stable for weaners when they weigh 27 kg (0,14 vol%). N_2O measurements indicate the growth with animal age through concentrations in the range of 1,09 to 170 ppm (in average 42,34 ppm) in fattening and 1,1 to 63,33 ppm (in average 21,29 ppm) in weaning stables.

CO_2, NH_3 in N_2O concentrations and microclimate parameters in stables air significantly (P<0,05) depend to ventilation rates, concentrations in exhaust air significantly (P<0,05) depend to ventilation rates as well. Average concentrations from exhaust air from fattening stables were following: CO_2 0,13 vol%, NH_3 8,0 ppm, CH_4 0,00, N_2O 52,9 ppm, beside average concentrations in exhaust air from weaning stables indicate: CO_2 0,19 vol%, NH_3 6,82 ppm, CH_4 0,00, N_2O 36,92 ppm

Calculation considering ventilation rates of total emissions from 21 stables used for pig fattening and pig weaning has shown that CH_4 emissions can be measured just in few cases, meanwhile emissions of N_2O arise with the age of animals and achieve more than 2000 ppm in the period when pigs reach the weights around 100 kg. After this period emissions of N_2O decline to lower levels. Regarding enlarged ventilation rates and hotter outdoor climate influence in ahead of this period this fact is comprehensible however emissions of N_2O were probably still arising. In weaning stables N_2O emissions did not reach 1000 ppm what can be explained due to younger animals and their weight. On the other hand emissions of NH_3 from fattening and weaning stables were arising with animal weight constantly. Figure 2 show representative results of CO_2, NH_3, CH_4, N_2O emissions emissions from pig fattening and weaning stables.

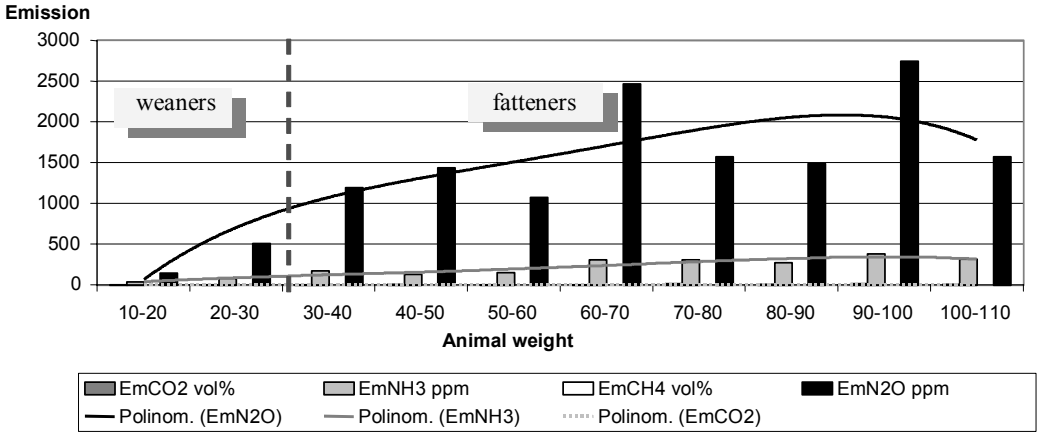

Figure 2. CO_2, NH_3, CH_4, N_2O emissions from pig fattening and weaning stables

CONCLUSIONS

In generally results represent lower greenhouse gases emissions from broiler production, especially N_2O concentrations in comparison to layer-egg production. Moreover lower emissions of greenhouse gases were monitored from pig-weaner production such as in pig-fattening production, as well, where N_2O emissions in some cases were extremely high. We can consider differences in emissions among stables due to farm technology, animal production phases, number, weight and age of animals like in poultry so as in pig production. We can report enormous N_2O concentrations in some pig-fattening stables, and low infrequent appearance of CH_4 emission. In majority we consider significant ($P<0,05$) microclimate changes due to variable air streams in stables, directly correlated to N_2O concentrations in the air. Air stream in fan exhausters was responsible to significant ($P<0,05$) changes in CO_2, NH_3 in N_2O concentrations on openings, where air exhausts from fan exhausters. Methane (CH_4) can be established just in few cases.

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ODOURS IDENTIFICATION IN THE LAYING HEN'S EXPERIMENTAL ROOM AND THE USE OF ALUMINOSILICATES TO DEODORIZATION

Opaliński, S. and Dobrzański, Z.

Wroclaw University of Environmental and Life Sciences, Poland

SUMMARY

In the air of the experimental room (vivarium) for laying hens 29 different organic compounds were identified using gas chromatography methods. The concentrations of sulphur organic compounds were between 0,043 and 56,360 $\mu g \cdot m^{-3}$ and the concentrations of other organic compounds were between 0,464 and 111,713 $\mu g \cdot m^{-3}$. The air of the vivarium was purifying with the use of filtering machine (prototype) filled with sorbents (aluminosilicates). The reduction rate of sulphur organic compounds was on the level of 43,4% for bentonite and 59,7% for halloysite. The reduction rate of other identified organic compounds was 26,0% for both sorbents.

INTRODUCTION

Livestock buildings are responsible for enormous emission of gaseous pollutants [2, 7, 16]. For example, in air of the swine confinement buildings more over 160 chemical compounds have been identified [3]. Odours, like phenols, indoles, scatols, thiols, sulphides, aliphatic amines and VFA as well as ammonia emitted from animal faeces can affect both animals and human health [4, 6, 8, 17]. Ammonia emission from agriculture sources is more than 90% of total NH_3 emission and the half of that originates from livestock and related activities [1, 18]. We cannot ignore the fact that emission of aerial pollutants from buildings for intensive animal production represents serious environmental risk. Moreover, elimination of aerial pollutants could have both economic and animal welfare benefits.

To reduce gaseous pollution from livestock buildings we can decrease theirs concentration by filtrating the air inside the buildings or by filtrating the outlet air. We can also reduce it by adding sorbents to bedding material. In both cases the use of aluminosilicates sorbents seems to be a very good solution. It is possible to place sorbents into filtrating machine as a filter bed as well as putting it straight into the litter. The aluminosilicates are the class of clay minerals which have very big sorptive capacity and which are both inexpensive and easy available. Besides, the aluminosilicates do not cause any harmful effects on animals and humans, furthermore their presence in animal excreta increase the value of manure [5, 10, 11].

The aim of this work was to assess the concentrations of organic compounds in the air of the experimental room for laying hens and also to examine the method of the deodorization.

MATERIALS AND METHODS

The investigation was made in the experimental room (vivarium) for laying hens, where 120 laying hens (ISA SHAVER) were kept in the battery system (battery with 3 floors). The

conditions in the experimental room met the requirements (i.e. temperature and relative humidity) for laying hens.

The prototype of filtering machine (fot.1) filled with the aluminosilicates sorbents, bentonite or halloysite, were used to purify the air in the experimental room. The time of air purification was 4 hours; the samples of air were collected before and after the use of filtering machine. Each of the sorbents was testing during 7 days. Additionally, the samples of air were also collected during the period of 7 days (blank week) when the air of the experimental room was not purifying.

The samples of air were collected to the Tedlar bags, at height of 1 meter above the floor, with the use of electronic pump (NS-512). The organic compounds from the sample were concentrated by adsorption on the Carbotrap 400 and than after thermal desorption, were dosage into the gas chromatograph HP 5890 with the flame ionization detector (FID) or with the flame-photometric detector (FPD).

RESULTS AND DISCUSSION

During the research, 29 different organic compounds were determined in the air of the experimental room. The concentrations of the sulphur organic compounds (tab.1) were between 0,0430 $\mu g \cdot m^{-3}$ for dipropyl sulphide and 56,360 $\mu g \cdot m^{-3}$ for methanethiol. The concentrations of other 21 identified organic compounds (tab. 2) were between 0,464 $\mu g \cdot m^{-3}$ for 1-butanol and 111,713 $\mu g \cdot m^{-3}$ for 1-pentanol. None of the samples that were examined contained such organic compounds like 1,2,4,5-tetramethylbenzene, dodecane, naphthalene, 1-butanethiol, dimethyl disulphide and carbon disulphide.

The total concentrations of organic compounds were between 69,234 and 331,614 $\mu g \cdot m^{-3}$ and the total concentrations of sulphur organic compounds were between 35,968 and 179,219 $\mu g \cdot m^{-3}$. The range of the total concentrations determined in our investigation was corresponding with the results of other authors. For example, in the air samples from poultry house (7000 laying hens) Tymczyna et al. [12] determined the average total concentration of sulphur organic compounds on the level of 422,8 $\mu g \cdot m^{-3}$ during the spring period and on the level of 248,1 $\mu g \cdot m^{-3}$ during the summer period.

The total concentrations of both organic and sulphur organic compounds in the air samples were always lower after than before the purification of the air in the experimental room. However, the total concentration of the sulphur organic compounds for the blank week was lower than in the period of experiment when halloysite was testing. That could be the effect of microclimate changes inside of the experimental room. The reduction rate of sulphur organic compounds was on the level of 43,4% for bentonite and 59,7% for halloysite. For other organic compounds the reduction rate was on the level of 26% for both tested sorbents. The results clearly prove that the applied aluminosilicates sorbents and the method of their use were efficient in limitation of odours.

The levels of reduction rates for organic compounds determined by many authors were comparable with our results. For example, Tymczyna et al. [14] were purifying the air from hatchery hall with the use of prototype biofilter. The reduction rate for all identified sulphur organic compounds was on the level of 51%. However, the reduction rate for methylethyl sulphide was on the level of 69%, and the highest reduction rates (but not statistically significant) were for ethanethiol and methanethiol. The same authors [15] were using biofilter to optimize the air from poultry farm. The reduction rates obtained for some of the sulphur organic compounds

were very high, for methanethiol and ethanethiol the reduction rates were on the level of 74% and for diethyl sulphide even 97%. The use of the prototype open biofilter to purify the outlet air from layer house was also investigated [13]. The average reduction rate for alcohols, aldehydes, aliphatic and aromatic hydrocarbons was on the level of 30% during the 180 days of the experiment. However, better reduction rates were determined for individual compounds. For ethanol, pentanal, penthyloamine and indole the reduction was on the level of 50% and for xylene and trichloroethylene even over 60%. Sheridan et al. [9] applied the biofilter filled with shavings to purify the outlet air from the piggery. The odour's limitation was very efficient and the reduction rate was between 88 and 95%.

It was very important to notice that the most of the methods which were applied to limit the concentration of odours were concerning the outlet air of the livestock buildings. The prototype filtering machine that was applied in our experiment was purifying the air inside of the building; therefore it was not only reducing emission of odours from the experimental room but also inside of it. Taking into consideration the animal and human health the type of filtering process that was examined in our experiment seem to be a better solution.

CONCLUSIONS

The problem of odours and their emission from livestock buildings is still unsolved. Many scientists is paying attention to the process of deodorization, looking for the best and the most efficient method of elimination of gaseous pollutants from animal production. The method investigated in our experiment is on the beginning of the elaboration but the obtained results look promising and require further examination to ensure that the use of sorbents is not only moving the emission problem from livestock building up or down the chain.

ACKNOWLEDGEMENTS

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Table 1. The concentrations of the sulphur organic compounds ($\mu g \cdot m^{-3}$) in the samples of air collected before and after the use of sorbents and also during the blank week

The name of compound	Blank week	The name of sorbent			
		Haloysite		**Bentonite**	
		before	after	before	after
Sulphur dioxide	18,575	11,483	6,693	3,562	1,868
Carbon oxysulphide	29,297	3,939	n.d.	5,951	7,166
Methanethiol	19,965	56,630	50,832	24,659	20,857
Ethanethiol	n.d.	40,453	41,235	n.d.	n.d.
Methylethyl sulphide	42,600	48,926	19,309	6,174	2,247
Diethyl sulphide	1,264	n.d.	n.d.	0,393	n.d.
Methylpropyl sulphide	1,427	0,866	0,488	0,914	0,419
Dipropyl sulphide	2,611	5,489	2,815	1,644	0,0430
Total concentration of organic compounds	**122,795**	**179,219**	**131,786**	**48,833**	**35,968**

n.d. – not detected

Table 2. The concentrations of the organic compounds (μg·m^{-3}) in the samples of air collected before and after the use of sorbents and also during the blank week

The name of compound	Blank week	The name of sorbent			
		Haloysite		**Bentonite**	
		before	after	before	after
Methane	7,538	5,894	5,605	8,713	6,215
Ethanol	2,917	16,491	n.d.	3,985	n.d.
2-butylamine	38,704	1,778	n.d.	n.d.	n.d.
Propanol	6,186	n.d.	n.d.	1,672	n.d.
Cyclobutanol	4,062	n.d.	n.d.	n.d.	n.d.
1-butanol	n.d.	n.d.	n.d.	n.d.	0,464
2-pentylamine	2,678	1,716	n.d.	3,213	1,879
Pentanal	2,499	n.d.	n.d.	n.d.	n.d.
2-methyl-1-propanol	15,175	37,192	1,541	n.d.	n.d.
Methylcyclopentan	17,132	n.d.	n.d.	n.d.	n.d.
Benzene	n.d.	10,715	5,662	9,711	5,723
Trichloroethylene	9,189	15,763	0,653	n.d.	n.d.
Toluene	3,530	3,701	2,637	2,423	2,38
Hexanal	3,715	11,941	7,955	9,036	8,529
1-penthanol	111,713	11,994	8,059	12,242	10,529
Indole	22,850	37,697	22,089	36,894	22,674
Ethylbenzene	56,794	17,722	11,822	6,223	2,483
M,p-xylene	3,929	8,997	7,575	6,067	4,349
o-xylene	2,929	0,952	0,666	10,115	n.d.
Phenol	2,911	9,861	3,165	9,137	4,009
3-caren	17,163	n.d.	n.d.	2,807	n.d.
Total concentration of organic compounds	**331,614**	**192,414**	**77,429**	**122,238**	**69,234**

Figure 1. Prototype of filtering machine

AIR QUALITY IN COW HOUSES WITH CURTAIN WALL VENTILATION IN FINNISH CLIMATE

Kivinen, T.[1], Mattila, K.M. [2], Teye, F.[2], Heikkinen, J.[3] and Heimonen, I.[3]

[1] MTT Agrifood Research Finland, Animal Production Research, Vakolantie 55, FI-03400 Vihti, Finland, tapani.kivinen@mtt.fi; [2] University of Helsinki, Department of Agrotechnology, Koetilantie 3, FI-00014 University of Helsinki, Finland, frederick.kwameteye@helsinki.fi; [3] VTT, Indoor climate and building services, Lämpömiehenkuja 2, FI-02044 VTT, Finland, jorma.heikkinen@vtt.fi, ismo.heimonen@vtt.fi

ABSTRACT

Most Finnish dairy barns – old and new ones – are ventilated with electric fans. Natural ventilation through wall curtains is a new technology in insulated dairy barns in Finland. The first equipment was assembled in 2004 and today it is expected to become a challenging option for dairy farmers. The main object of this research was to find out first how this new technology fits into Finnish climate and secondly what is the of indoor air quality performance. The research had mainly two phases. First a field measurement session was carried out in three selected insulated barns which had been built recently and equipped with curtain walls. The measurements took place between February and March 2006, a period for harsh frost. Instrumentation collected data from inside and outside temperature, relative humidity, wind and air velocity. Carbon dioxide and ammonia were recorded inside the barns as well as radiation and heat flow in the floor. Data was collected into computer data loggers. Secondly a year performance model was simulated according to the measured data. The model results reveal that curtain wall technology can be used in Finnish climate and it gives good air quality even in cold winter time air exchange situations. Summertime air quality is superb compare to electric fan solutions. The only negative points are a few frost days and high relative humidity inside the barn during wintertime.

CURTAIN WALL TECHNOLOGIES IN MEASURED BARNS

The field measurements were done in three cow houses in middle Finland. The oldest barn had been one year in operation and the youngest was brand new. The barns contained 120–140 cubicles. The floor area varied between 960 and 1900 m^2. Wall curtains in all cases were hand adjusted by farmers' intuition. The farmers made a book keeping about curtain openings so that this data was used in the final calculations. The year simulation was made according to animal heat, humidity, CO_2 production, ammonia production, milk yield and forage intake values. U-values in constructions were considered as they were built. Solar radiation, local long time climatic data were added into the formula.

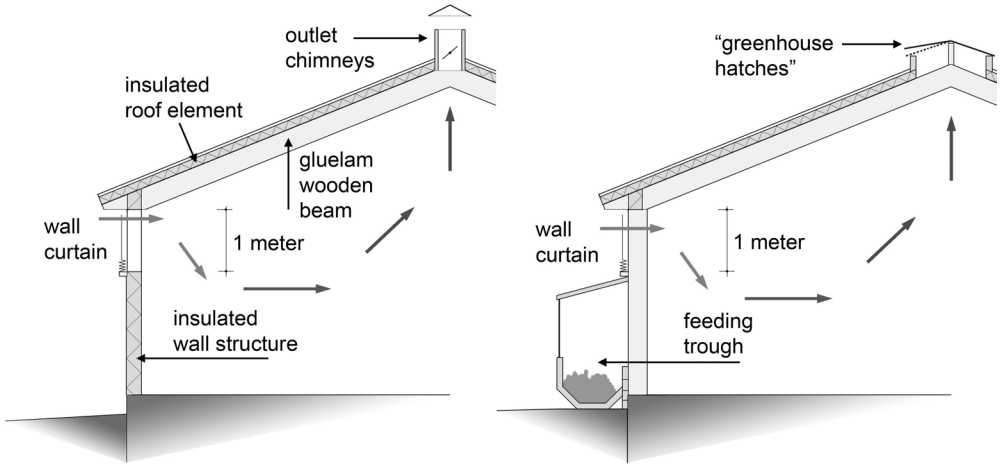

Figure 1. Ventilation inlet and outlet technologies in measured barns. In all cases walls and roof were insulated, the inlet opening was one meter (when fully open) and outlet openings had several types, chimneys and the whole ridge long greenhouse hatches

YEAR SIMULATION FOR VENTILATION AND AIR QUALITY

The year simulation was calculated with a computer program created by VTT researchers. The simulation was based partly to the measured data and a long term weather data from Finnish meteorological institute. The simulation numbers were calculated for a barn with 124 cubicles, floor area 1245 m^2 and volume 6350 m^3. The simulation barn had 80 milking cows and 30 heifers and dry cows. The total continuous energy produced by the animals was 130 kW. The walls and roof were thermal insulated. The floor had no insulation at all.

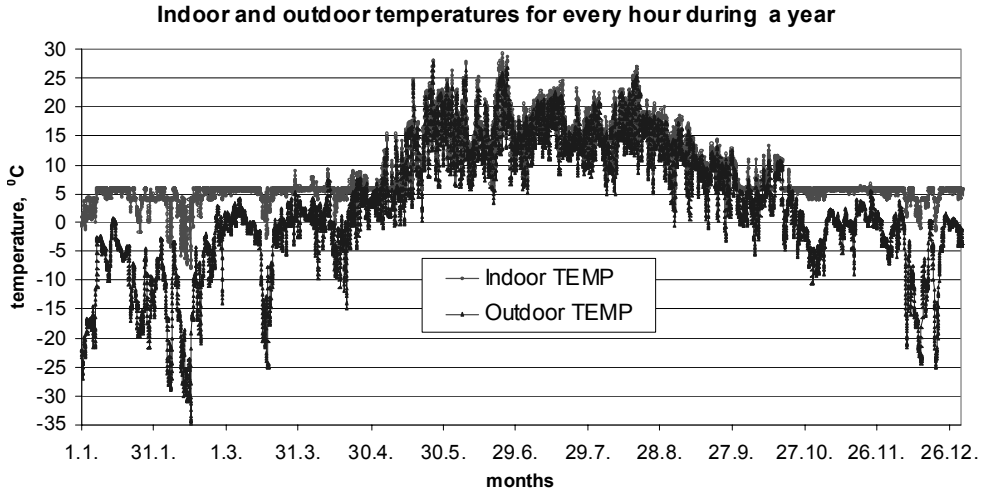

Figure 2. Indoor temperatures according to simulation

The inside temperature will be controlled to maintain +4 – +6°C level through autumn, winter and spring. For a few days in January – February the inside temperature may go slightly under 0°C due to extreme outdoor winter temperatures. The additional heating is needed under –23°C outdoor temperature if the farmer wanted to keep the inside temperature over 0 C. Practically however indoor surfaces, drinking cup or water hoses do not freeze even if the additional heating didn't exist. Summertime indoor temperatures follow outdoor temperatures.

Carbon dioxide is a rather good indicator for air quality and air exchange rate. The simulation indicates that CO_2 concentration is very acceptable. The maximum level in Finland is 3000 ppm. It seems to exceed the limit only for 3,4% of all 8760 hours a year during the coldest periods in wintertime when the ventilation is at its minimum. But in return the level remains mostly at 500 ppm for nearly 6 months a year.

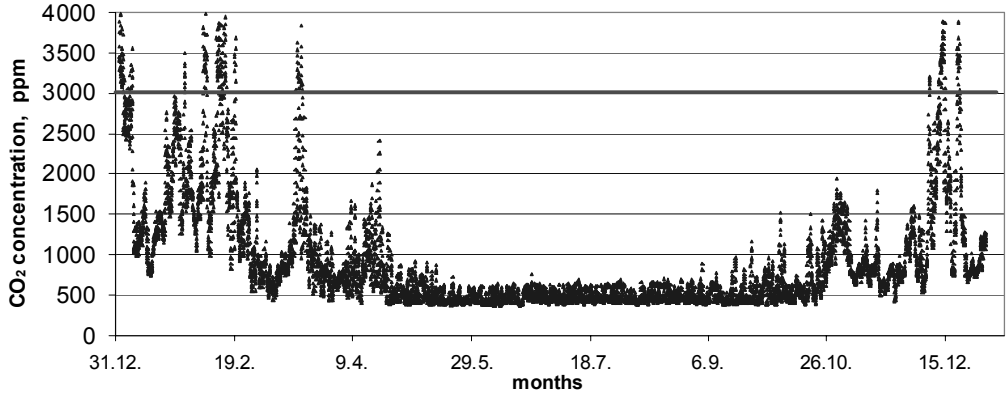

Figure 3. Carbon dioxide concentration according to simulation

The relative indoor humidity is hard to be controlled if judged according to CIGR recommendation for a dairy barn. Relative humidity occurs in intensively during the winter time, sometimes reaching 100% (dots over 100, in figure 4) and then the air becomes foggy. But the relative humidity exceeds the CIGR's 90-rule also between +5 and + 20°C which are springtime, autumn and summer temperatures as well. At the same time the barn can be too dry. This means that the barn is dependent on outdoor circumstances due to summertime day and night variation of temperature and humidity. But still the high relative humidity can be a threat to constructions especially for timber and steel. It also can cause mildew growth and premature decay on wood surfaces.

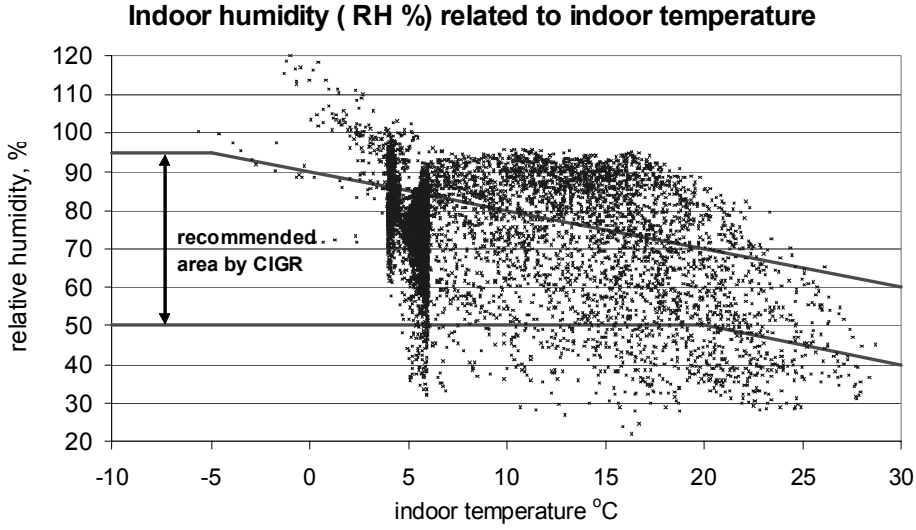

Figure 4. Indoor humidity according to simulation

Technical assembly and detailing of the curtain makes the system vulnerable for air leakages. These leakages are not harmful. On the contrary they maintain the sufficient level of basic air exchange during the cold winter times when the curtain is closed. This feature practically keeps the minimum ventilation rate in proper level.

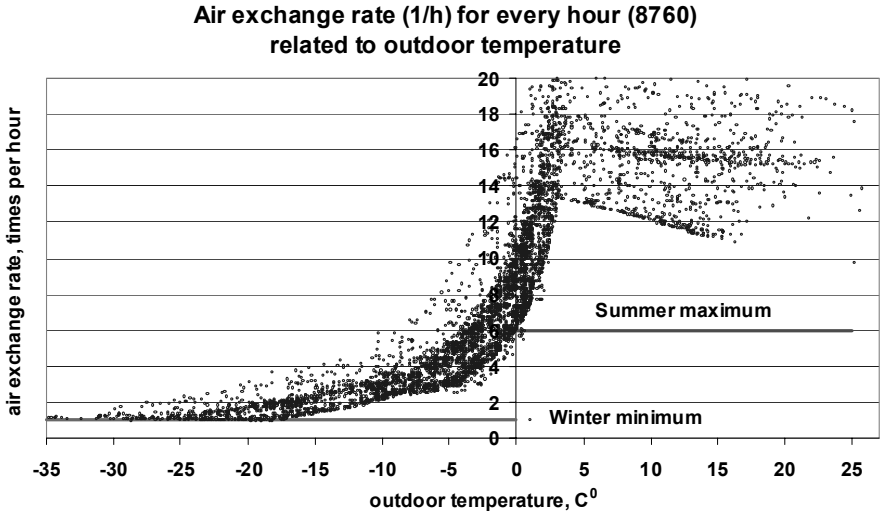

Figure 5. Air exchange rate

The blue dots in figure 5 indicate the air exchange rate (times per hour) related to outdoor temperature. The winter minimum and summer maximum lines are indicate the ventilation rate requirements in Finnish legislation for animal husbandry buildings in agriculture. The winter minimum exchange rate can be achieved even in the coldest periods. When the outdoor temperature reaches 0 C air moves and changes 6–10 times per hour. On a calm summer day air changes approximately 12 times and even 50–100 times on a windy day. The wall curtain system always guarantees minimum exchange and the maximum rate is well beyond requirement. This gives a good air exchange rate and thus appropriate air quality.

CONCLUSIONS

The curtain wall system appears to be an acceptable and functional ventilation concept for insulated dairy barns in Finnish climate if few inconveniences are accepted. These are slight frost days under zero and high relative humidity. In return the ideal temperature + 5°C can be maintained over 7 months in Finnish climate. Gas emissions are always in control and minimum air exchange is good and maximum exchange very good. The system also consumes very little electric power. If the barn of this size had electric fans the annual energy consumption could otherwise be 16000 kWh. The system is also rather silent. These features give a good hygienic production environment for cows in air quality and acoustic environment.

POSTER PRESENTATIONS

THE EFFICIENCY OF A SLAUGHTERHOUSE WASTEWATERS TREATMENT PLANT AND THEIR POLLUTANT POTENTIAL

Borda, C.

University of Agricultural Sciences and Veterinary Medicine, Faculty of Veterinary Medicine, Hygiene and Environmental Protection Dept., 3–5 Manastur St., 400372 – Cluj-Napoca, Romania cborda@usamvcluj.ro

SUMMARY

The pollutant potential of residual waters from slaughterhouses can be decreased by multiple purification methods. Researches undertaken in a modern chicken slaughterhouse, equipped with a mechanical-biological purification station and with final evacuation in surface waters, showed that it did not function properly. Thus, in spite of the fact that measured parameters reduced with values between 4.27 and 100%, the maximum admitted limits of pH, ammonium and CCO-Cr were exceeded.

Keywords: wastewater, slaughterhouse, efficiency, pollutant potential

INTRODUCTION

Because of their composition (Borda and Drăghici, 2001), residual waters from slaughterhouses have a high pollutant potential. In order to treat these waters and decrease the pollution, many technologies exist, with proper results if they are applied correctly.

Our previous researches showed that the small studied slaughterhouses are not properly equipped for the treatment of wastewaters. As a consequence these waters pollute either the sewage networks (Borda et al., 2002), or the surface water in which they are discharged (Borda et al., 2005).

As a result of Romania's adhesion to the European Community, many slaughterhouses were closed, because they did not respect the veterinary sanitary standards and environmental protection.

The present work aims to establish the efficiency of wastewater treatment plant from a modern poultry slaughterhouse as well as the pollutant potential of wastewaters at the discharge point.

MATERIALS AND METHODS

The research was undertaken in a slaughterhouse with a maximum capacity of 24,000 tones/year, the volume of residual waters being of 350–400 m^3/day. The slaughterhouse is provided with a modern mechanical-biological treatment plant, the circuit of the raw wastewater is as follows:

plumes skimmer – primary settling tank – releasing sieve – homogenisation tank – flocculator – polymers added tank – floating unit – aeration tank**.** After treating the water being evacuated in surface water (spring). Samples were collected in two points: at the entrance of water in the treatment plant (raw wastewater) and at the entrance of water in the spring (treated wastewater).

Four determinations in one year were made, and the following parameters were analysed:

- sediment – with Imhoff cones;
- conductivity – with electronic conductivity-meter (Conmet 1, Hanna Instr.);
- pH – with electronic pH-meter (Checker 1, Hanna Instr.);
- dry matter – at 105 ^{o}C, after centrifugation;
- ammonium – by distilation;
- chemical oxygen demand – potassium bicromate method;
- biochemical oxygen demand – Winkler method;
- total number of aerobic mesophilic germs (TNAMG) – with nutrient agar;
- most probable number of total coliforms and fecal coliforms – the multiple test tubes method, with lactose broth for the presumptive test, and with Levine medium for the confirmation of total coliforms and brilliant bile broth for the confirmation of fecal coliforms.

RESULTS AND DISCUSSION

The results of the analyses being presented in the following tables:

Table 1. Physical and chemical parameters

Parameter	**Sample**	**1.**	**2.**	**3.**	**4.**
Sediment	RW	8.2	5.5	2	0.5
(mL/L)	TW	0	0	0	0
	D%	**–100**	**–100**	**–100**	**–100**
Conductivity	RW	1346	404	306	1169
(µS/cm)	TW	1038	1239	1047	947
	D%	**–22.88**	**+206.68**	**+242.15**	**–18.99**
pH	RW	5.80	5.52	6.31	6.17
	TW	4.94	4.43	6.04	5.60
	D%	**–14.82**	**–19.74**	**–4.27**	**–9.23**
Dry matter (mg/L)	RW	810.41	927.08	625	1666.66
	TW	525	647.05	520	208.33
	D%	**–35.21**	**–30.20**	**–16.8**	**–87.50**
Ammonium (mg/L)	RW	95.78	14.00	21.61	48.24
	TW	18.15	12.20	14.40	0.90
	D%	**–81.05**	**–12.85**	**–33.36**	**–98.13**
COD-Cr (mgO_2/L)	RW	1120.00	1323	630.00	584.00
	TW	99.00	45	138.00	79.00
	D%	**–91.16**	**–96.59**	**–78.09**	**–86.47**
BOD_5	RW	225.00	322.00	94.50	118.50
(mgO_2/L)	TW	12.10	8.45	14.90	5.50
	D%	**–94.62**	**–97.37**	**–84.23**	**–95.35**

RW-raw wastewater; TW-treated wastewater; D% – percentage differences between raw and treated wastewater.

Table 2. Bacteriological parameters

Parameter	Sample	1.	2.	3.	4.
TNAMG (cfu/mL)	RW	74,000	105,000	1,810,000	225,500
	TW	6775	1103	5800	76,000
	D%	**–90.84**	**–98.94**	**–99.67**	**–66.29**
Total coliforms (MPN/100 mL)	RW	3,300,000	33,000	2,600,000	17,200,000
	TW	16,090	3300	7900	278,000
	D%	**–99.51**	**–90**	**–99.69**	**–98.38**
Faecal coliforms (MPN/100mL)	RW	3,300,000	33,000	1,700,000	17,200,000
	TW	16,090	3300	4900	278,000
	D%	**–99.51**	**–90**	**–99.71**	**–98.38**

After the water crossed the treatment plant, the values of determined parameter reduced, in almost all cases:

- sediment totally reduces in all determinations;
- conductivity reduces its values at the first and last determination, in the other 2 cases increasing with 200%;
- pH reduces with percentages between 4.27 and 19.74;
- dry matter reduces in the 4 determinations with percentages between 16.8 and 87.50;
- COD-Cr reduces with values between 78.09 and 96.59%;
- BOD_5 reduces with percentages between 84.23 and 97.37;
- the most dramatic decrease is that of bacteriological parameters between 90 and 99.71%.

In spite of this fact, the admitted limits for used water evacuated in natural receptors (NTPA-001, 2002) exceeded for the following parameters:

- pH, in all 4 determinations, with values between 0.47 and 2.07 pH units (figure 1.);
- ammonium, in the first three determinations, with values between 9.2 and 15.15 mg/L (figure 2.);
- COD-Cr, in determination no. 3, with 13 mg/L (figure 3.).

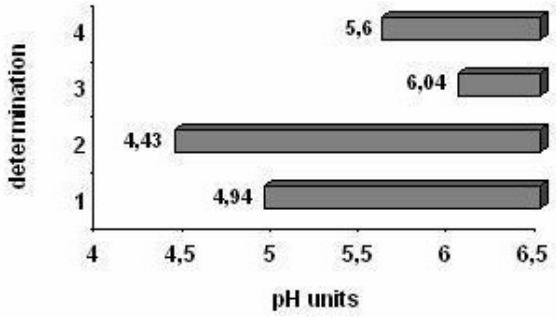

Figure 1. pH-exceeded of lover admissible limit

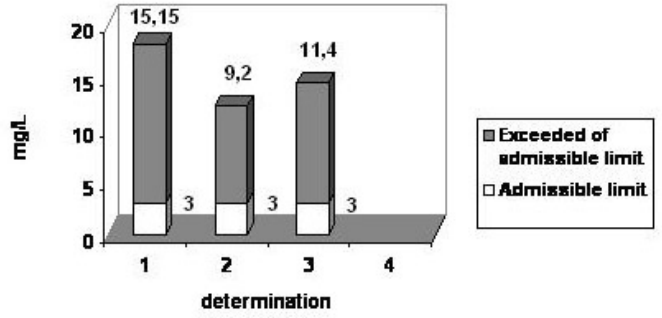

Figure 2. Ammonium-exceeded of admissible limit

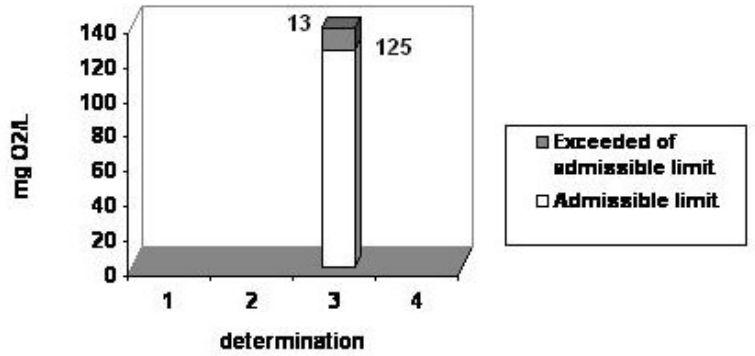

Figure 3. CCO-Cr-exceeded of admissible limit

CONCLUSIONS

After analysing all these results, the conclusions are:

- the treatment plant from this slaughterhouse, even if it is modern, is not very efficient and produce the pollution of surface waters in which used water is evacuated; this fact is due, in my opinion, to a break in the functioning of the slaughterhouse, produced shortly after its first use;
- urgent optimization measures are needed, in order to stop the pollution.

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THE OCCURRENCE OF POTENTIALLY PATHOGENIC BACTERIA IN ATMOSPHERIC AIR IN THE VICINITY OF A SEWAGE TREATMENT PLANT

Breza-Boruta, B. and Paluszak, Z.

Department of Microbiology, University of Technology and Life Sciences Bernardyńska 6/8, 85–029 Bydgoszcz, Poland, mikro@utp.edu.pl

SUMMARY

The aim of the study was to evaluate the microbiological contamination of atmospheric air on the premises and in the vicinity of the Municipal Sewage Treatment Plant in the town of Słupsk. The concentration of selected bacteria in atmospheric air was determined at the fermentation pools, at the composting facility as well as 350, 600, and 800 m outside the sewage treatment plant. The largest bioaerosol emission source in the sewage treatment plant was the storage area of maturing compost. The total number of bacteria reached the maximum of 10^4 CFU m^{-3}. The number of the indicator bacteria *Pseudomonas fluorescens* ranged from 0 to 14.3 CFU m^{-3}. *Escherichia coli* was detected only in one time in May.

On the basis of the results obtained one may come to the conclusion that the tested facility does not pose a hazard of the emission of tested bacteria over the areas beyond the sewage treatment plant.

Keywords: bioaerosol, *Pseudomonas fluorescens*, *Escherichia coli*, sewage treatment plant

INTRODUCTION

Municipal objects have an effect on surrounding soils, surface and ground waters, the air, and through the atmosphere, on distant agriculture, town and recreation areas. Main factors contaminating the air are chemical and microbiological (bioaerosols) pollutants and odours [1, 6]. Bioaerosols are mainly created by opportunistic pathogens commonly occurring in soil and water. They are mainly bacteria of the genera: *Pseudomonas*, *Enterobacter* and *Bacillus* [9]. A large number of microorganisms occurring in the air is a frequent indicator of a bad sanitary state of the environment surrounding big urban agglomerations. Air may be a way of transmission of microbiological pollutants from contaminated areas to not contaminated ones. Particular attention should be given to ways of the air flow and the elimination of pollution sources [1].

Sewage is a potential carrier of pathogenic microorganisms and it may pose a health hazard when it penetrates into the atmosphere during aeration [2]. Sewage aeration using an aerator, diffuser, sprinkles and water-drawing wheels increases the probability of transmission of microorganisms present in sewage into the air [5].

Monitoring studies are frequently conducted recently, concerning the measurements of the air composition. The knowledge obtained makes it possible to design new sewage treatment plants properly and improve their exploitation so as to minimize the noxious effect on the environment.

The aim of the study was to estimate the impact of the Municipal Sewage Treatment Plant in Słupsk on the level of air microbiological contamination and to determine the range of spreading the emitted bacterial aerosol.

MATERIAL AND METHODS

Monitoring studies of atmospheric air were conducted at the Municipal Sewage Treatment Plant in Słupsk and at points located at different distances from the object: point 1 – composting facility, point 2 – open fermentation pools, point 3– a farm about 350 m away to the east and about 600m to the south from the sewage treatment plant, point 4– agricultural and orchard area about 800 m away to the east from the sewage treatment plant.

The air samples were taken with the crash method using Microbiological Air Sampler MAS-100 Eco™ by Merck. Through the head of the apparatus a strictly determined air volume was sucked on a Petri dish with agar medium, according to a season of the year and atmospheric conditions. The research was conducted monthly in the period from October to December 2005 and from March to June 2006.

The following groups of microorganisms were determined at suitable selective media:

- total bacteria number on standard nutrient agar (incubation at 28ºC – 72 h)
- *Pseudomonas fluorescenc* on the King B medium (incubation at 28ºC – 48h)
- *Escherichia coli* on agar ENDO with fuchsine and lactose (incubation at 37ºC – 24 h)

All air measurements for the tested groups of microorganisms were made in four replications. Meteorological conditions during the sample collection were presented in Table 1.

On the basis of the microorganism number obtained the means were calculated from the colony-making units. In order to work out the results, a conversion table of the positive holes number for the air monitoring system MAS-100 was applied, and the colony number obtained was counted over 1 m^3 of the atmospheric air. The evaluation of the atmospheric air pollution level was performed according to the recommendation given in the Polish Standards: PN-89/Z-04111/02 [8]

RESULTS AND DISCUSSION

The number of the tested microorganisms in atmospheric air turned out to vary depending on a place and time of sampling. The results, showing the bacteria number and the level of air pollution were presented in Fig. 1 and 2 and in Table 2.

The total number of bacteria in the air on premises of the tested sewage treatment plant ranged from 10^1 to 10^4 $CFUm^{-3}$ (Fig. 1.). The highest bacteria emission took place at the composting facility. At this site a strong air pollution occurred in May, and the total number of the bacteria reached up to 17220 CFU m^{-3}. At the fermentation pools and at all the points beyond the sewage treatment plant air pollution did not occur. The total bacteria concentration determined at these points indicates that the air is not polluted according to the Polish Standards recommendations.

Table 1. Meteorological conditions during air sampling (data coming from IMiGW in Słupsk)

Sampling date	Pressure (hPa)	Mean temperature (ºC)	Humidity (%)	Wind direction	Wind velocity (m/s)
18.10.2005	1025.8	7.1	87	N	2
14.11.2005	1017.0	7.1	87	W	1
05.12.2005	999.7	2.8	90	SE	2
02.03.2006	996.7	–1.6	83	SW	2
04.04.2006	1008.3	4.6	79	SSE	2
08.05.2006	1019.3	13.9	66	N	3
02.06.2006	1024.2	10.5	77	NW	2

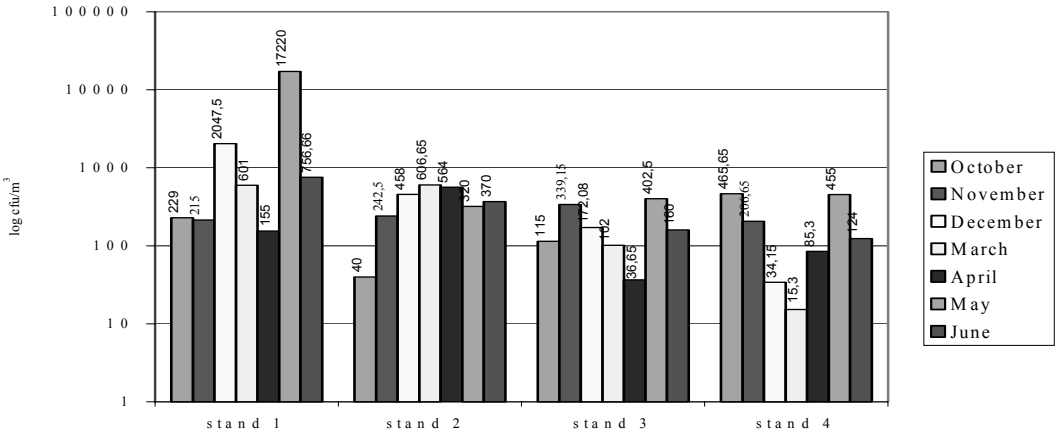

Figure 1. Total number of bacteria at particular sites in autumn 2005 and spring 2006

In the case of the indicator bacteria *Pseudomonas fluorescens* the higher concentration coming to 14.25 $CFUm^{-3}$ was also reported in the air at the composting facility (Fig. 2). The bacteria were the cause of moderate air pollution at the open fermentation pools during the whole research period. At sites 3 and 4 outside the sewage treatment plant their number decreased from 4.5 to 0 $CFUm^{-3}$ along with a distance from the object. At the point at a distance of 800 m from the sewage treatment plant *P. fluorescens* was not recorded in two times: in December and April.

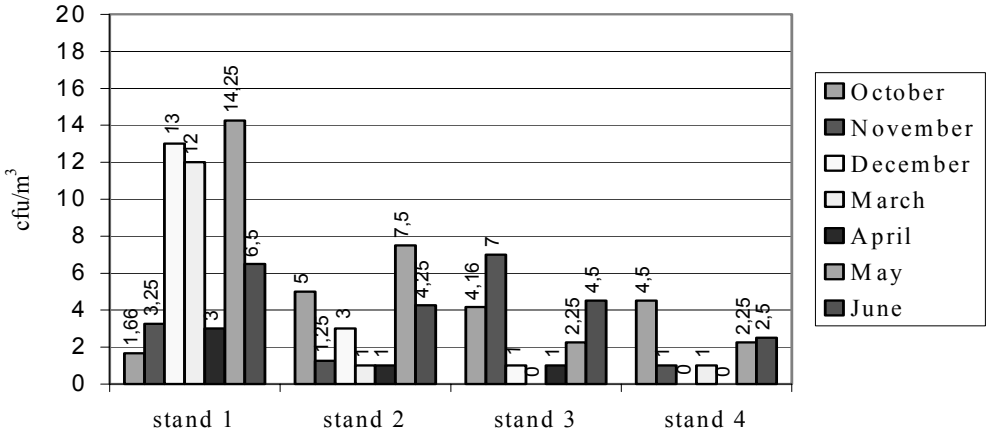

Figure 2. Number of *Pseudomonas fluorescens* at particular sites in autumn 2005 and spring 2006

During the whole research period from October to December 2005 and from March to June 2006, *E. coli* was detected only in May in the air at two sites: at the composting facility and 350 m outside the sewage treatment plant (Table 2). No *E. coli* was reported in the air at the open fermentation pools as well as at a distance of 600 and 800 m outside of sewage treatment plant.

Table 2. Number of *Escherichia coli* in autumn 2005 and spring 2006 at the tested sites

Stand	Concentration [CFU m $^{-3}$]						
	October 2005	November 2005	December 2005	March 2006	April 2006	May 2006	June 2006
I	0	0	0	0	0	2	0
II	0	0	0	0	0	0	0
III	0	0	0	0	0	2	0
IV	0	0	0	0	0	0	0

The level of bacterial bioaerosol concentration was diverse at particular measurement points. It depended mainly on a distance from the sewage treatment plant, but also on atmospheric conditions (wind direction), and a season of the year. Bioaerosol is spread mainly with the wind in eastern direction. The highest level of microorganisms was observed in May. In the studies by Petrycka [6] and Pillai [7] it was observed that a high temperature may contribute to the increase in emission of potentially pathogenic microorganisms.

The results obtained proved that the number of microorganisms penetrating into air decreases substantially along with a distance from the source of emission. This is supported by the research conducted by other authors [2, 4].

For the bacteria of the family *Enterobacteriaceae* there are no standards indicating the admissible content of this groups of bacteria in the air. Analyses conducted at German composting plants indicated a considerably higher level of *E. coli* than in the present study. Böhm [3] reports that a number of the bacteria of the genera *Escherichia coli* on the premises of the composting plant amounted even to 2400 CFUm^{-3} air.

Only the microorganisms which are the most resistant and best adapted to unfavourable living condition maintain longest their viability in the air [10]. One should bear in mind that bacteria

contaminating atmospheric air may also come from other sources than the sewage treatment plant. A lot of factors have an effect on the number of microorganisms in the air, such as the lie of the land, or tree-covered areas [1].

CONCLUSIONS

1. The composting facility was the biggest emitter of bioaerosol at the sewage treatment plant. Most tested bacteria were isolated here.
2. No significant generation of bacterial bioaerosol was detected from the open fermentation pools, which is confirmed by a low concentration of the tested bacteria in the air.
3. The bacteria *Pseudomonas fluorescenes* occurred at all the research sites for most time of the study. Their absence was reported at site 3 in March and at site 4 in December and April. Their number shows that the air was moderately contaminated with this species of bacteria.
4. Potentially pathogenic bacteria *Escherichia coli* were detected only in one time at sites 1 and 3. The results obtained at the points located in different distances and direction from the sewage treatment plant indicate that the tested facility does not influence negatively on the microbiological quality of the air around the area and does not pose a threat to local people, and the applied technology of sewage treatment does not contribute to spreading the tested microorganisms.

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BIOLOGICAL POLLUTANTS OF AIR IN POULTRY HATCHERY HALL

Chmielowiec-Korzeniowska, A., Tymczyna, L., Drabik, A.

Department of Animal Hygiene and Environment, Faculty of Biology and Animal Breeding, University of Agriculture in Lublin, Poland

SUMMARY

The egg-poultry industry, whose production technology is based on the biological material, constitutes a potential source of not only chemical pollutants but primarily of microorganisms and their toxins. These agents appear as a bioaerosol constituent, in particular of organic dusts. They are carried off through the ventilation installation outdoors, where they maintain at the high level up to one kilometre from the emission source. Numerous poultry diseases, among others the air-borne respiratory tract infections, may reach distant sites outlying even three kilometres from a place of their origin. It is hazardous not only for health of the plant workers but for the inhabitants of neighbouring areas.

The objective of the present work was to establish the biological pollutants concentration in the hatchery air. The researches were conducted in the Plants of Poultry Hatchery in Poland with the annual production output 20–25 mln chickens of meat hens Cobb and Ross lines.

As the present researches showed, the technological and engineering progress in the hatchery plants caused a substantial decrease of air contaminants, in that dust pollutants. Relatively slight dustiness assayed in the hatchery room averaged 1.0 mg/m^3. The studies proved that a mean concentration of bacteria in the hatchery room is 4.0 x 10^3 cfu/m^3 with a relatively high contribution of Gram-negative bacteria (nearly 6.5 x 10^2 cfu/m^3) and bacterial endotoxins (11.15 ng/m^3). Among them the following bacteria were identified: *Acinetobacter, Citrobacter, Enterobacter, Escherichia, Flavobacterium, Klebsiella, Pseudomonas, Leclerca, Sphingomonas, Xantomonas, Agrobacterium* and *Pantoea*.

Keywords: dust, bioaerosol, hatchery, endotoxin

OBJECTIVE

The animal breeding environment constitutes a perfect „laboratory" for microbe generation and multiplication. The egg-dairy industry whose production technology is based on the biological material also makes a potential source of not only the chemical pollutants but, or primarily, microorganisms and their toxins(among others, endotoxins).These agents occur as a bioaerosol constituent, mainly of organic dusts. They are vented through the ventilation installation outdoors where they persist at the high level up to a kilometre from the emission source. A number of poultry diseases, among others the air-borne respiratory tract infections are drifted over long distances, even up to three kilometres from the place of their origin [Schlegemilch, 2005; Seedorf, 1998; Wathes, 1998b].That pose a serious health threat not only for the plant workers but the inhabitants of the near-by region.

An air microbe count is one of the major index of confined space contamination. However, it is hard to estimate a bioaerosol concentration owing to a great number of parameters affecting

directly or indirectly the aeromicroflora count. The finer bioaerosol particulates, the deeper they deposit in the respiratory system and thus appear more difficult to eliminate from organism at exhalation. An infection depends not only on a particulate size but the microorganism count and kind as well. A bioaerosol influence on the higher organisms is subject to a microbial count in the air, their quality, dispersion level as well as solid and liquid particulates serving as a condensation nucleus for microorganisms [Wathes,1998a; 1998b].

The objective of the present work was to establish the biological pollutants concentration in the hatchery air.

METHODS

The study was conducted at the Poultry Hatchery in Dębówka, 20km south of Warsaw, Poland. The hatchery has an annual output of 20 to 25 million Cobb and Ross meat hens, which represents 4% of the national production. Five series of experiments were carried out in the hatchery room, which was equipped with 8 hatchers (AS-4H, Petersime, Zulte, Belgium) and 12 incubators (AS-4S, Petersime, Zulte, Belgium) with an input of 115th eggs. In each series of experiments, 4 air samples were collected in 2 place (A and B) in the hatchery room. A – point in the centre hall, B point in the outlet ventilation system.

In the air samples, concentrations of dust and microbial pollutants as well as endotoxins were determined. Dustiness was established by weight method, whereas a bacteria concentration and species composition using a filter method with surface inoculation on the following agar media:

- blood agar to determine total bacteria count and mesophylic actinomycetes
- eosin methylene blue (EMB) agar to identify Gram-negative bacteria
- tryptic soy (TS) agar with half reduced nutrients to determine thermophylic actinomycetes.

The microbe colonies incubated on each medium were assayed macro- and microscopically first after the Gram staining method. Afterwards, a number of morphological types was established and their concentration in 1 m3 of the air sample was estimated and expressed as colony forming unit – cfu/m3.The bacterial isolates were identified using the biochemical assays API 20E and API 20 NE (bioMerieux, Marcy l`Etoile, France) for the Gram- negative bacteria and Gram-positive cocci. Mesophylic actinomycetes were determined by the micro- and macroscopic methods.

An endotoxin concentration was measured using the *Limulus* test kit (bioMerieux, Marcy l`Etoile, France).

The pollutants concentrations at the site A were compared to those at the site B using the Wilcoxon nonparametric test.

RESULTS

The present researches confirmed that the technical and engineering progress in the hatchery plants has substantially reduced air dust pollutants. In the hatchery hall, there were determined rather low dustiness levels that averaged 0,95 cfu/m^3 in the livestock buildings [Baykov and Stoyanov, 1999; Chang 2001; Kalingan, 2004; Wathes, 1998a; Zucker, 2000].That indicates lesser hazard of the respiratory system diseases incidence in the workers employed there and in turn, lower burden of the outdoor environment.

In the hatchery halls, alike the poultry houses, the dusts comprise fine particulates of faeces, feathers and epidermis introduced to the halls along with the fertilized eggs. They are also produced at chick hatching. The organic pollutants get into the productive environment together with fungi and their spores, bacteria and their toxins (endotoxins), viruses and parasites. The performed researches revealed a mean concentration of bacteria at the level of 4 thousand cfu/m^3 in the hatchery hall, including Gram-negative and positive bacteria, cocci and Gram-positive rods as well as actinomycetes. The investigated air samples exhibited predominance of staphylococci and streptococci (Streptococcus faecalis in particular) –Gram-negative cocci that averaged over 3 thousand cfu/m^3. A bioaerosol composition studied in the hatchery room showed a relatively high content (16%) of the Gram-negative bacteria with mean concentration of 651 cfu/m^3. Among them, beside sporadically reported pathogens *Salmonella* genus, the presence of enteric rods (*Enterobacteriaceae*), aerobic rods of *Alcaligenes* and *Acinetobacter* genera was confirmed. The studies of the other authors indicate that these bacteria often constitute the microflora of air in the livestock buildings. They are widely distributed in soil, water and in the bacterial microflora of healthy human skin (mainly *A. johnsonii*, *A. lwoffii*, *A. radioresistens*). More rarely these bacteria colonize the gastrointestinal tract and nasopharyngeal cavity. The bacteria of *Acinetobacter* most often develop pneumonia, sepsis, urinary tract infection, dermatitis or wound infection. It is most likely that the bacteria of this genus make up one of the major sources of endotoxins penetrating the hatchery air.

A mean concentration of bacterial endotoxins in the hatchery hall air reached 11,5 ng/m^3. According to Rylander [1977], this level is a potential risk factor of the respiratory system disease incidence in human exposed to it. The Dutch Expert Committee on Occupational Standards recommended the maximum allowable exposure limits for endotoxins at 8-hour working time at 4.5 ng//m^3 level, that is twofold lower compared to that reported above [Heederick and Douwes, 1997]. As the inspection of the hatchery plants revealed, this standard is very difficult or just impossible to comply with.

The statistical analysis did not show any differences between the pollutants concentration determined at the air sampling sites (for $p \leq 0,05$).

CONCLUSIONS

The studies of the bioaerosol composition at the hatchery hall showed a relatively high percentage of the Gram-negative bacteria and their toxins (endotoxins). The disinfection procedures practiced in such plants do improve the sanitation status of the rooms and reduce microorganism count, yet they do not eliminate their residuals, i.e. endotoxins.

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Table 1. Concentration of dusts, endotoxins and bacteria in the hatchery hall air

Group/type		**Total**		**A Hall centre**		**B Ventilating device outlet**	
		M	**SD**	**M**	**SD**	**M**	**SD**
Dust [mg/m^3]		0,95	1,29	0,51	0,34	1,39	1,78
Endotoxin [ng/m^3]		11,15	17,12	11,03	19,18	11,27	17,08
Bacteria [cfu/m^3] including:		4051,85	5439,00	3785,20	7155,34	4318,50	3896,70
G (–) rods	*Pseudomonas putida*	651,45	723,99	475,20	560,95	827,70	887,16
	Pseudomonas sp.						
	Pseudomonas vesicularis						
	Salmonella spp.						
	Sphingomonas paucimobilis						
	Sphingomonas multivorum						
	Acinetobacter baumannii						
	Acinetobacter lwoffii						
	Agrobacterium radiobacter						
	Enterobacter cloacae						
	Escherichia coli						
	Flavobacterium meningosepticum						
	Leclercia adecarboxylata						
	Citrobacter youngae						
Gram (+) cocci	*Enterococcus faecalis*	3292,10	5050,47	3525,80	6824,45	3058,40	3268,27
	Micrococcus spp.						
	Staphylococcus spp.						
Gram (+) rods	*Brevibacterium otitidis*	85,4	214,75	25,4	52,94	145,4	290,19
	Brevibacterium spp.						
	Corynebacterium spp.						
Gram (+) bacilli	*Bacillus spp.*	13,75	33,71	22,00	45,23	5,50	9,86
Mesophylic actinomycetes	*Streptomyces albus*	6,15	9,02	6,80	7,86	5,50	10,95
	Streptomyces spp.						
Thermophylic actinomycetes	*Thermoactinomyces thalpophilus*	3,00	6,75	0,00	0,00	6,00	8,94

A – hall center, B – vent air outlet

VALIDATION OF MEAT BY-PRODUCTS COMPOSTING PROCESS ON THE BASIS OF THE INACTIVATION OF SELECTED MICROORGANISMS AND PARASITE EGGS

Ligocka, A., Paluszak, Z.

Department of Microbiology, Faculty of Agriculture, University of Technology and Life Science, 85-029 Bydgoszcz, ul. Bernardyńska 6; mikro@utp.edu.pl

SUMMARY

Animal by-products of category 3 can be transformed without pre-treatment in a composting plant to produce compost. In order to minimize a risk to human health and environmental hazard not only should the microbiological supervision of the final product be carried out, but the validation of composting process should be implemented as well. This allows the evaluation of a particular technology in killing pathogens introduced into raw material which undergoes the process of composting. Test organisms should be exposed in a similar matrix as treated material. In this experiment *Salmonella senftenberg* W_{775}, BPV and ECBO viruses and *Ascaris suum* eggs were applied for validation of the method. Two composting cycles with different maximal temperature were analyzed.

Keywords: animal by-product, *Salmonella, Ascaris*, BPV, ECBO, compost

INTRODUCTION

Meat waste management is a serious ecological problem in all EU member states. Although they are rich in organic matter with a high fertilizing value, at the same time they can contain numerous pathogenic microorganisms (bacteria, viruses), mainly of enteric origin, and nematodes and their ova. The microorganisms most frequently isolated from meat waste are bacteria of the genera *Salmonella*, *Escherichia*, *Clostridium*, and faecal *Streptococci* of D group. There can also occur pathogenic viruses, such as: viruses ROTA, bovine parvovirus (BPV), bovine enterovirus (ECBO), and the ova of the parasite *Ascaris suum*. For epidemiological reasons only the waste subjected to previous sanitization can be used for agricultural purposes. Meat waste can be hygienized by pasteurization, stabilization in termophilic condition, liming and composting. According to Straub (11) the temperature of 55–65^{o}C is sufficient for the elimination of pathogens contained in composted material. According to Strauch (12) unfavourable antagonistic environmental conditions have bigger influence on pathogenic microorganisms than a higher temperature itself. The notable effect of this research was the evaluation of the time and conditions needed for meat waste utilization process which guarantee the elimination of noxious microorganisms. This permitted us both to recycle vast amount of waste effectively, and to eliminate sanitary and epidemiological hazard resulting from using the products obtained in agriculture, so it will contribute greatly to the improvement of environmental safety.

MATERIAL AND METHODS

In the experiments a bio-reactor was used with a mixing drum which was loaded with the meat batch previously prepared in a device grinding and mixing meat waste (stomach contents, scraps, fats, blood) – 60% with a texturizer (sawdust) – 40%. The carriers with pathogens were placed in front, centre and back of the bioreactor. Additionally a wire baskets containing indicator pathogens were put in composting biomass, it was remained there till the end of composting process (5 days). Bone carriers included fragments of shafts and distal parts of thighbones of pigs. Bone marrow was removed from them and polycarbonate bags were placed inside, containing 10^{7-8} cfu/ml of bacteria suspension, and also filters with adsorbed viruses. Pores with a diameter of 0.01 nm in the polycarbonate foil allowed the contact of microorganisms with composted biomass. Meat carriers were particles of pork closed in cubes made of metal netting measuring 3 by 3 cm and 5 by 5 cm, which were to protect them from deformation during composting. Bacteria suspension was injected inside of the particles. Large meat carriers and bone heads, due to small recesses in the bio-reactor were placed only in a capsule inside the appliance and analyzed only at the last time of the process. In theory they were supposed to protect the bacterial suspension best from thermal conditions in the bio-reactor. The carriers were removed from the biomass in several-hour intervals and the number of streptococci was determined based on the MPN method in 3-test tube set. Two multiplying media will be used in the process of *S. senftenberg* W_{775} isolation. At the first stage weighed portions will be placed in 1% peptonic water (incubation at 37°C for 24 hours), and then 0.1 ml material will be transferred from each test tube to a line of test tubes containing 10 ml selective liquid medium following Rappaport (incubation at 43°C for 24 hours). Next the material will be sieved to selective agar medium BPLA following Kaufmann (incubation at 37°C for 24 hours). Final identification will involve using serological tests – polyvalent serum HM.

The method of viruses' determination was based on the Filter-Sandwich principle. 1 ml of virus suspension (BPV – bovine parvovirus and ECBO – enteric cytopathogen bovine orphan virus) with a titre of 10^6 – 10^8 $TCID_{50}$/ml in phosphate buffer with pH 6.5 was placed on a Virosorb-Zetapor membrane. Membranes containing the carriers were placed in polycarbonate bags with pores of 0.01-μm diameter, that prevented virus particles from penetrating beyond the carrier, and then they were placed in appropriately prepared bone and meat carriers – in bones the filters were surrounded with bone marrow, and a meat carrier was minced meat placed inside of a plexiglass container. The carriers were placed in the bio-reactor and then removed at definite intervals and the titres of viruses that did not undergo inactivation during composting were determined. Virological tests were also carried out on the base of inactivation of the suspension of viruses occurring in Eppendorf test tubes. The test tubes were placed in a composting facility. Examination of BPV and ECBO viruses' titres was conducted on cell lines MDBK with the microplate method. Strain suspensions in elution medium were diluted in the Eagle minimal essential medium in logarithmic progression (10^{-6}–10^{-8}). 0.05 ml of appropriately diluted virus suspension, 0.05 ml of the minimal essential medium and 0.1 ml of a cell line was added to each of four wells in microplates. The microplates were incubated in a thermostat at 37°C. Infected cultures were observed on day 4 and 5 under the microscope. The titres were calculated with the method of Karber and presented as $TCID_{50}$/ml.

Infective eggs were prepared from adult *A. suum* worms collected from pigs at slaughter. The eggs were obtained from the proximal 2 cm of uterus by dissection of female worms. Thereafter nylon bags with eggs (20 μm pore size) were placed into biomass. Determination of *Ascaris suum* eggs survival - eggs after removing from the bags were placed in a Petri dish with sterile distilled

water and incubated during 30 days at 30°C. After this time the percentage of live eggs from which larvae developed was counted under the microscope.

RESULTS

The research comprised two cycles of composting. The temperature of the biomass was monitored continuously. In cycle I it ranged from 50 to 60°C and was similar at all points where the carriers were placed, while in cycle II it reached the highest values at the back part of the appliance, yet not exceeding 50°C (Fig. 1).

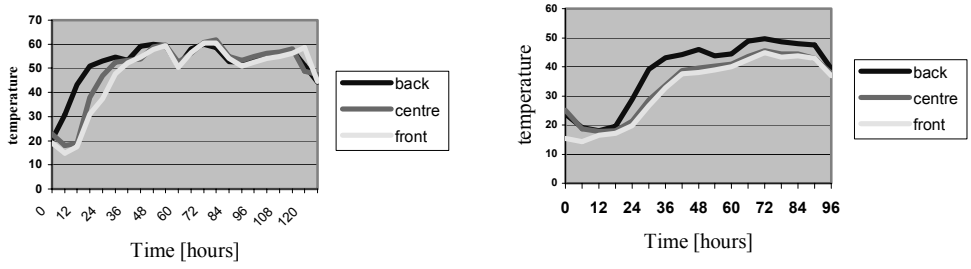


Figure 1. Temperature of composted biomass during cycle I (A) and II (B)

The high temperature of the biomass in the cycle resulted in the fast elimination of the bacteria. They were not detected in the bone carrier in the 48th hour, and in the meat carrier in the 72nd hour of the process (Fig. 2).

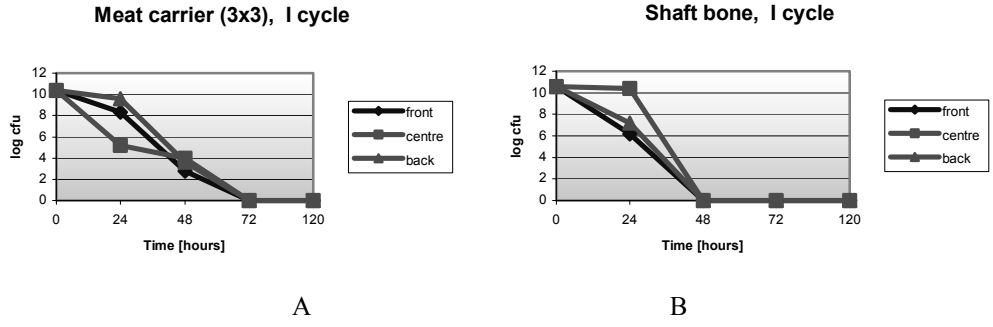
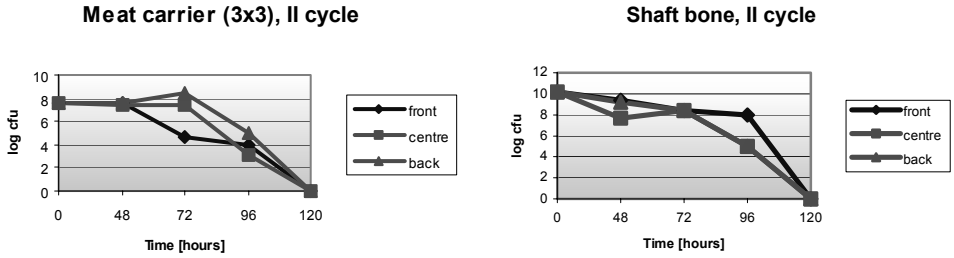


Figure 2. Survival of the bacteria in a small meat particle (A) and shaft bone (B) during cycle I

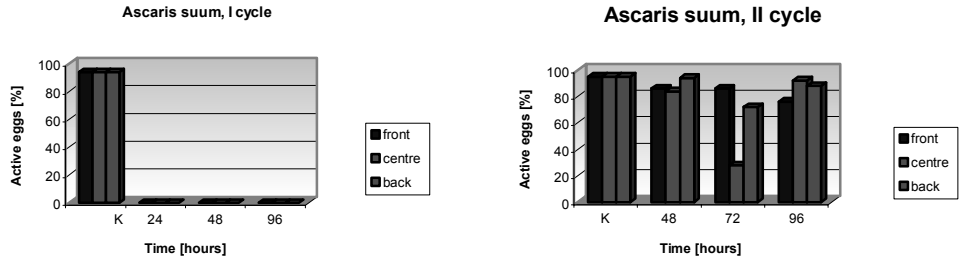
In cycle II, the elimination rate of the microorganisms was remarkably slower than in cycle I. Both in the meat carrier and bone carrier the inactivation of rods proceeded only in the fifth day of composting. Besides, a minor multiplication of the bacteria occurred during the process (Fig. 3).



A B

Figure 3. Survival of the bacteria in a small meat particle (A) and shaft bone (B) during cycle II

In respect of the survival of *A. suum* eggs only cycle I proceeded effectively. Viable eggs were not isolated as early as after 24 hours of composting, while in cycle II, almost the identical percentage of invasive eggs as in the control was still noted in the 96th hour (Fig. 4).



A B

Figure 4. Inactivation of *Ascaris suum* eggs in cycle I (A) and II (B) in different parts of the composting device

S. senftenberg were not isolated from the largest carriers placed inside the biomass after 120 hours. Thus theoretically, the best protected bacteria, placed in the largest carriers, undergo equally efficient elimination in both cycles of composting (Fig. 5).

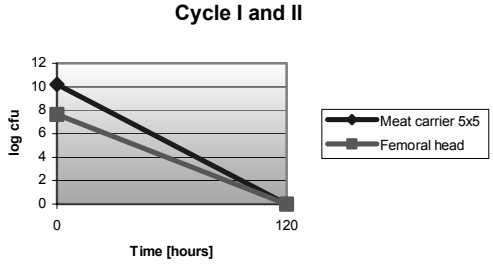


Figure 5. Survival of the bacteria in a big meat carrier 5x5 and a head of thighbone placed in the big carrier during both of cycles.

The thermoresistant virus BPV was still detected in the carriers in the 72 hour of composting. It was subject to the fastest reduction in the bone carrier (a decrease in titre by 2 units already in the 48th hour), while in the eppendorf tubes its titre practically remained at the same level (Tab. 1). The ECBO virus studied in the cycle with a lower temperature underwent a weakest inactivation in the meat carrier, while in bone and eppendorf a decrease in titre by almost 3 log was observed.

Table 1. Titre of the virus BPV in three different times of cycle I

Carrier	Time (hours)			
	0	24	48	72
	Virus titres [$TCID_{50}$/ml]			
meat (filters)	5.30	5.20	4.80	3.81
bones(filters)	5.30	4.55	<1.55	<1.92
Eppendorfs	6.15	6.42	6.17	5.55

Table 2. Titre of the virus ECBO in four different times of cycle II

Carrier	Time (hours)				
	0	48	72	96	120
	Virus titres [$TCID_{50}$/ml]				
meat (filters)	4.40	4.05	3.80	3.55	-
bones(filters)	4.40	3.80	<1.55	<1.55	-
eppendorfs	5.70	5.70	4.80	4.42	2.80

– carrier damaged

DISCUSSION

Of the applied methods for meat waste utilization, composting is gaining an increasing popularity. A properly handled composting process results in an effective pathogen elimination (effectively eliminates pathogens as a result of) due to the self-heating of composting biomass (4, 14). When the development of the thermophilic stage cannot be obtained, the composted material may pose a risk of spreading microorganisms in the environment. For a proper hygienization of composted material, the process should proceed for at least 3–4 weeks, and in addition for 1 week the temperature in the composted biomass should amount at least 65^{o}C (13). In the present studies in cycle 1 the temperature ranged between 50 and 60^{o}C, while in cycle II it did not exceed 50^{o}C, which was reflected in the differences in bacteria and parasite egg survival. Namely, after 72 hours in cycle I bacteria elimination in both the meat and bone carriers was obtained, while at the same time in cycle II the number of *Salmonella* sp. was almost identical in comparison with the control. Due to the environmental hazard, the inactivation of rods is one of the basic measures of utilization efficiency of waste intended for agricultural use. This sort of experiments was conducted in Germany, where *Salmonella senftenberg* W_{775} was the indicator organism (3, 15). This serotype rarely causes infections in humans, while is characterized by a remarkable resistance to high temperatures. In the experiment by Jepsen et al. (5) the number of *Salmonella*, which amounted to 3600 cells in 100 g of raw sewage sludge, in a sample of ready compost of the same weight was reduced to 2 cells. The microorganism elimination rate in the composted material depends mainly on a temperature and the time of its action, though an increasing role is

also attributed to the activity of native microorganisms. Their particular importance consists in the ability to inhibit the process of *Salmonella* re-multiplication in biomass (10).

Also *Ascaris suum* eggs are commonly applied to studies on evaluation of organic waste hygienization efficiency, as they are easily available, as well as highly resistant to environmental factors (17). Veerannan (16) indicated that in low temperatures sewage sludge are not free of invasive eggs until after 3 years of storage. Only in thermophilic conditions the full elimination of the eggs takes place (7, 8). Composting method ensures the full waste hygienization and parasite eggs inactivation on condition that all the biomass is subject to the temperature of 55^{o}C for 2 weeks or 65^{o}C for one week (14). Similar results were obtained in the present studies. The inactivation of *Ascaris suum* eggs proceeded more effectively in cycle I. The presence of viable eggs were not already recorded after 24 hours, while in cycle II, after 96 hours they still made almost 100% of all the population placed in the carrier (like in the control).

Viruses are determined in organic wastes definitely less frequently than other pathogens due to the lack of simple methods for their isolating in contaminated samples. The current state of the research suggests that the influence of the environment on virus inactivation is multiform. Apart from the temperature, an increased content of ammonia has also an effect on their elimination in waste (18), heavy metals (1) as well as the proteases and nucleases of microorganisms. The effect of temperature on the elimination rate of enteroviruses was studied by numerous authors. Within the range 70-80^{o}C they were inactivated after 1-2 minutes and at 70^{o}C - after 10 minutes (9). Enteroviruses are inactivated at a temperature of 60^{o}C already after 15 minutes, and at 54^{o}C - after 45 minutes (8). Rehmnan (9) reported that a temperature of 50^{o}C is not sufficient to inactivate the virus ECBO, though it significantly decreases its titre In the present studies, the virus ECBO placed in the biomass of the cycle with a lower temperature underwent the weakest inactivation in the meat carrier (0.85 log), while in the bone and eppendorf we observed a decrease in titre by nearly 3 log. The virus BPV is characterized by termostability in the moist heat of 75^{o} to 90^{o} (2). Due to its extraordinary resistance against moist heat, parvoviruses proved to be well-suited for checking chemo-thermal and thermal disinfection procedures. Monteith and Shannon (6) studied the inactivation of enteric viruses by composting cattle faeces. The parvovirus was seeded into the solid fraction of cattle manure. The operating temperatures reported in this study were 30° C on day 1, 45° C on day 2 and 60° C for the rest of the experiment. The results showed that the parvovirus did not survive composting for 28 days. In the present study physical and chemical parameters were not sufficient for the total elimination of the virus BPV. It underwent the fastest reduction in the bone carrier (a decrease in titre by 2 units as early as in the 48th hour), while in the eppendorf its titre practically remained at the same level.

CONCLUSIONS

1. Mistakes are made during waste utilization process, resulting in an improper hygienization of the biomass. The effect of this is that large quantities of parasite eggs may penetrate into the environment, posing a hazard for human and animal health. The application of the indirect control of the process based on introduction of indicator bacteria, parasite eggs and viruses into the biomass seems to be necessary for ensuring the environmental biosafety.
2. Varied temperatures in the bio-reactor in two analyzed cycles caused the difference in the elimination rate of *S. senftenberg* W_{775} and *A. suum* eggs in the carriers tested.

3. During a properly conducted composting process, the elimination of the bacteria and *A. suum* eggs occurred irrespective of the size of carriers used.
4. Thermo-resistant viruses (BPV) underwent reduction only in the bone carriers. The inactivation rate in the other carriers did not exceed 1.4 log. The ECBO virus underwent a weakest inactivation in the meat carrier, while in bone and eppendorf a decrease in titre by almost 3 log was observed.
5. The study indicates that the elimination of *Salmonella* and *A. suum* eggs is possible in the case of temperature reduction from 70^{o}C to 55-60^{o}C, on condition that it has a long-term effect on biomass.

ACKNOWLEDGEMENT

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AIR POLLUTION WITH SULFUR COMPOUNDS IN RABBIT HOUSE AND FOX FARM

Nowakowicz-Dębek, B., Saba, L., Wnuk, W., Martyna, J. and Chmielowiec-Korzeniowska, A.

Laboratory of Reproduction Biology of The Department of Animal and Environment Hygiene, The Faculty of Biology and Animal Breeding UA in Lublin, ul. Akademicka 13, 20-950 Lublin, POLAND

SUMMARY

The air analysis for a sulphur compounds content was performed in the rabbit house (4,55 livestock unit (UL) and in the fox farm (2 UL).The air samples were collected to the Tedlar bags at the height of the animal cages. The research showed the presence of 14 sulphur compounds in the air of both farms. A higher concentration of sulphur compounds was recorded in the rabbit farm where carbon disulphide at the level of 622,63 μg/m^3 prevailed significantly. In the fox farm, however, the highest values were noted for phenols (118,43 μg/m^3) and indoles (21,03 μg/m^3). Deficiency of such compounds like, carbonyl sulphide, carbon disulphide and methylpropyl sulphide may point to some differences in the metabolism of herbivorous and carnivorous animals or different microclimatic conditions.

Keywords: rabbit, polar fox, air pollution, sulphur compounds

INTRODUCTION

Atmospheric air is a mixture of gases and its composition in the breeding objects clearly undergoes some changes closely connected with a species of animals maintained, their nutrition, management system etc. Volatile organic pollutants are released to the air directly by the animals or indirectly from the accumulated organic matter. To preserve a typical flavour of the air in the livestock buildings is an alternative for measurable economical profits as well as a necessity to conform to the domestic and the EU requirements [1, 3, 7, 8].

The objective of the present work was monitoring and identification of sulphur compounds emitted to the air in the polar fox farm and rabbit house (the objects of different management systems).

METHODS

The air examination was carried out in the polar fox farm and rabbit house situated in the southern part of Poland. Throughout the research period, in the rabbit house about 650 animals as the basic herd were housed (4,55 LU), while at the fox farm – 50 fox vixens as the basic herd [2 LU].

In the warren the animals were kept in the one-story cages produced according to Ferm-Stal system. In the breeding pavilion, a natural ventilation and mechanical exhaust is used.

At the fox farm, the animals are caged in the pavilion system. In both objects the faeces were removed every day and heaped up outdoors to be used for the agrotechnical practices.

The air for examination was collected twice to the Tedlar bags at the height of animals' cages (ca 130 cm) in the fox farm and rabbit house. The obtained samples were analysed chromatographically using the permeate models and appropriate analytical software [2]. At the same time the following basic microclimatic parameters were measured: temperature, moisture, air movement [5].The obtained data were analyzed statistically and compared in the Tables.

RESULTS AND DISCUSSION

A significant factor for the animal maintenance proves to be their state of equilibrium with the internal environment they stay at. Homeostasis constitutes a vital condition for the organism functioning as well as productive benefits for their breeders. Therefore, monitoring the quality of each breeding environment factor is of primary importance, particularly the sulphur compounds regarded as noxious odours, odorforming and hazardous [4,6,9].

The chromatographic analysis of the air from the rabbit house and fox farm exhibited the presence of 14 sulphur compounds (Tab.1). In the rabbit house, three higher levels of sulphur compounds were determined. Carbon disulphide prevailed significantly as it reached a level of 622,63 $\mu g/m^3$, while among the mercaptans the highest concentrations were detected for methyl mercaptan (99,85 $\mu g/m^3$) and ethyl (95,47 $\mu g/m^3$). This high concentration of the sulphur compounds pollutants in the rabbit house air is extremely hazardous in the case of poor ventilation and low oxygen supply. Then some opiate-like by-effects may occur. The rates of these compounds emission have been exceeded in both, rabbit house and fox farm.

Absence of such compounds like carbonyl sulphide, carbon disulfide and methylpropyl sulphide at the fox farm may arise from a different composition of feedstuff supplied to the animals that is differences in metabolism of herbivores and carnivores. In the fox farms, the released volatile organic compounds undergo the direct transformations under the presence of light and other gaseous admixtures. Their spread is closely connected with the local climatic conditions.

In both farms, the permissible upper limits for phenol and indol (MPL – 2,5 $\mu g/m^3$) [10] were surpassed. The presence of identified pollutants in the air at the studied objects may have a negative influence on the health status of animals kept there. A direct or indirect mode of these substances penetration into organism means their getting into the circulation system and in turn, dissimilation all over body. As a consequence, they are accumulated in the tissues, disturb the organs and systems functioning that result in the animal performance decrease.

The air quality monitoring went along with the assessment of microclimatic parameters that showed noticeable differences between them, i.e., temperature, moisture and air movement (Tab.2). The air temperature in the fox farm appeared to be lower as compared to the rabbit house and averaged 13,2^{o}C and 18,8^{o}C, respectively. Relative moisture value was higher in the fox farm – mean 75%, while in the rabbit house – 66%. An air movement rate in the fox farm reached 2,60 m/s and 0,15 m/s in the rabbit house. The obtained values were found within the minimum limits of the zoohygienic standards for the animals maintained in the livestock buildings (0,10–0,30 m/s).

CONCLUSION

The pollutants released in the rabbit house differed quantitatively and qualitatively compared to the fox farm which confirms the differences in these species metabolism. However, very high levels of volatile organic pollutants determined in the rabbit house imply the poor ventilation system and that may decrease the animal performance quality.

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Table 1. Mean levels of sulphur compounds subject in farm of rabbit and foxes (μg/m^3)

Gaseous compounds	Animal maintenance section	
	Rabbit	Foxes
	$\bar{x}$ ±SD	$\bar{x}$ ±SD
hydrogen sulphide	9,73±6,32	1,53±1,73
sulphur dioxide	8,3±2,14	2,52±1,49
carbonyl sulphide	46,72±25,12	n.d.
methyl mercaptan	99,85±31,60	0,05±0,09
Ethyl mercaptan	95,47±43,10	0,07±0,11
carbon disulphide	622,63±112,4	n.d.
isopropyl mercaptan	45,25±13,8	0,10±0,19
methyl ethyl sulphide	3,92±0,98	1,48±2,13
diethyl sulphide	0,50±0,23	0,67±0,60
methylpropyl sulphide	0,22±0,10	n.d.
dipropyl sulphide	1,06±0,84	1,69±1,81
phenol	4,80±1,15	18,43±18,2
indole	35,60±22,5	21,03±15,6
metyl sulphide	n.d.	5,15±6,05

Notes: $\bar{x}$ – arithmetic mean; SD – standard deviatian; n.d. – not detected

Table 2. Mean values of microclimatic parameters at rabbit and fox farm

Animal maintenance section	Air temperature (^{o}C)	Relative moisture (%)	Air movement (m/s)
Rabbits	18,8	66	0,15
Foxes	13,2	75	2,60

THE EFFECT OF COMPOSTING OF POULTRY EXCREMENTS ON THE SURVIVAL OF MODEL HELMINTH EGGS

Papajová, I.[1], Juriš, P.[1], Szabová, E.[1], Sasáková, N.[2], Venglovský, J.[2], Šefčíková, H.[1] and Vargová, M.[2]

[1] *Parasitological Institute of the Slovak Academy of Sciences, Hlinkova 3, 040 01 Košice, Slovak Republic; E-mail: papaj@saske.s;* [2] *University of Veterinary Medicine, Komenského 73, 041 81 Košice, Slovak Republic*

SUMMARY

The process of aerobic composting of poultry excrements was optimised under running conditions in composting pits by the changes in physical-chemical characteristics of the substrate. In composting piles, which were mixed so as that the resulting ratio of C:N would be above 15:1 and were regularly aired, the effect of aerobic composting of poultry excrements and straw on the survival and development of model non-embryonated *Ascaris suum* eggs was observed. Due to the changes in the pH value, C:N ratio, temperature up to 70°C, a decrease in the concentration of NH_4^+ as well as in the total nitrogen , 98.5±3.65 % of eggs were devitalised as soon as on day 4 from the beginning of composting. A total devitalisation of non-embryonated *A. suum* eggs occurred between day 4 and 7 of composting. Thus the risk of the dissemination risk, survival and potential spread of endoparasitic germs in the environment can be eliminated.

Keywords: composting, poultry excrements, *Ascaris suum* eggs, survival

INTRODUCTION

Animal manures have been used effectively as fertilizers for centuries. Poultry manure has long been recognized as perhaps the most desirable among these natural fertilizers because of its high nitrogen content. In addition, manures supply other essential plant nutrients and serve as a soil amendment by adding organic matter. The most serious problem is the liquidation of these organic wastes. Utilisation and disposal of wastes has been the subject of many investigations, which have described contamination of the environment with emissions, toxicity of treated wastes to plants, but also potential survival and spreading of pathogenic agents (Bernal et al., 1993; Juriš et al., 2000; Papajová et al., 2002 and others). In recent years, composting has been presented as an environmental friendly and sustainable alternative of how to manage and recycle organic solid wastes, with an aim to obtain a quality organic product to be used as organic amendment in agriculture (Pagans et al., 2006). The aims of this study was (i) to optimise the process of aerobic composting of poultry excrements under running conditions in composting piles by the changes in physical-chemical characteristics of the substrate and (ii) to monitor the effect of aerobic composting of poultry excrements and straw in composting pits on the survival and development of model non-embryonated *Ascaris suum* eggs.

MATERIAL AND METHODS

Poultry excrements and straw were used at the experiment. Basic characterisation of these materials is given in Table 1. Excrements collected from the poultry farm and straw were mixed and 6 piles (H1-H6) were built (1.5 m high, 5 m long and 2 m wide). The surface of the piles was not covered with any material to imitate natural conditions. The composting process at the piles proceeded from 17-day (H4-H6 piles) to 33–day (H1) retention time and in the thermophilic temperature range (to 70°C). Piles were periodically dug up (shovelled). The following changes in physical and chemical properties of the stored wastes were monitored: the pH, dry matter (DM), organic matter (OM), ammonium ions (NH_4^+), total nitrogen (N_t) and C:N ratio. The methods used corresponded to the STN 465 735. The C content was calculated according to the content of OM by the method of Navarro et al. (1993), and the C:N ratio was calculated.

Samples of poultry faeces were parasitologicaly examined using flotation techniques (Jurášek, Dubinský et al., 1993). We used the "artificial contamination of trough" with non-embryonated *A. suum* eggs approach to make sure that there was a sufficient number of positive samples in our observations. Eggs were inoculated into polyurethane carriers, prepared according to Plachý and Juriš (1995), at a dose of 1000 eggs per carrier. The carriers were placed to perforated PET bottles (50 ml) and introduced into a composting pile (H3). After the exposure in the pile, samples for parasitological and physical and chemical examination were collected after 0, 1, 2, 4, 7, 9, 14, 17 and 24 days of composting. Three samples were taken and analysed at the given sampling intervals. The controls with eggs were incubated in distilled water. Significance of differences between experimental and control groups were determined using Dunnet's multiple comparison test at the levels of significance of 0.05; 0.01; and 0.001 (Statistica 6.0). The physical and chemical properties of the organic material, as well as the number of devitalised non-embryonated *A. suum* eggs were expressed as the mean values±standard deviation ($\overline{x}$ ±SD).

Table 1. Physical and chemical properties of the basic (leasing) materials

	Straw	Poultry excrements
pH*	7.01	8.43
DM (%)	92.55	44.67
OM (%)	93.34	77.99
IM (%)	6.66	22.01
NH_4^+ (g.kg^{-1} DM)	<0.10	1.76
N_t (g.kg^{-1} DM)	9.80	36.93
C:N	48.52:1	11.53:1

*pH value in aqueous extract which was obtained by mechanically shaking the samples for 1 h with double distilled water at a solid: water ratio of 1:10 (dry weight/volume).
DM – dry matter, OM – organic matter, IM – inorganic matter, NH_4^+ – ammonium ions, N_t – total nitrogen

RESULTS AND DISCUSSION

Composting piles were mixed based on the laboratory results of the experiment. Piles were mixed so that the resulting ratio of C:N would be above 15:1 and regularly aired. The temperature changes during composting are show in Fig. 1. The physical and chemical properties of the composting material are given in Fig. 2–5. However, composted material contained a more

humified (stabilised) OM compared with the non-composted poultry manure. Our results correspond with the results of Tiquia and Tam (2002). Composting of poultry manure converted the soluble nutrients to more stable organic forms, thereby reducing their bioavailability and susceptibility to loss when applied to crop fields.

Poultry faeces were also parasitologicaly examined. No parasites were find at the symplex, therefore we used the "artificial contamination of trough" with non-embryonated *A. suum* eggs. These eggs are most resistant to environmental factors amongst the helminth eggs Thus they were chosen as the model. *A. suum* eggs were totally devitalised as early as between day 4 and 7 of composting process (Table 2) due to the high temperature and changes in physical and chemical properties of the composting materials during composting of poultry manure with straw.

Table 2. Damage of *A. suum* eggs during composting of poultry excrements

Exposure (days)	Damaged *A. suum* eggs ($\overline{x}$ %±SD)
0 (control)	16.02 ± 2.61
1	28.16 ± 1.18
2	58.26 ± 4.63*
3	69.11 ± 7.22**
4	98.50 ± 3.65**
7	100**
9	100**
14	100**
17	100**
24	100**

$\overline{x}$ – mean values, SD – standard deviation, *Significance at the level P<0.01, **Significance at the level P<0.001

We can conclude – from a parasitological point of view – that thermophilic aerobic composting had a lethal effect on the viability of helminth eggs. This way of treatment is thus not associated with a risk of dissemination, survival and potential spread of developmental stages of endoparasites to the environment via composted organic wastes. Output from the aerobic composting of poultry excrements and straw can be used as organic fertiliser.

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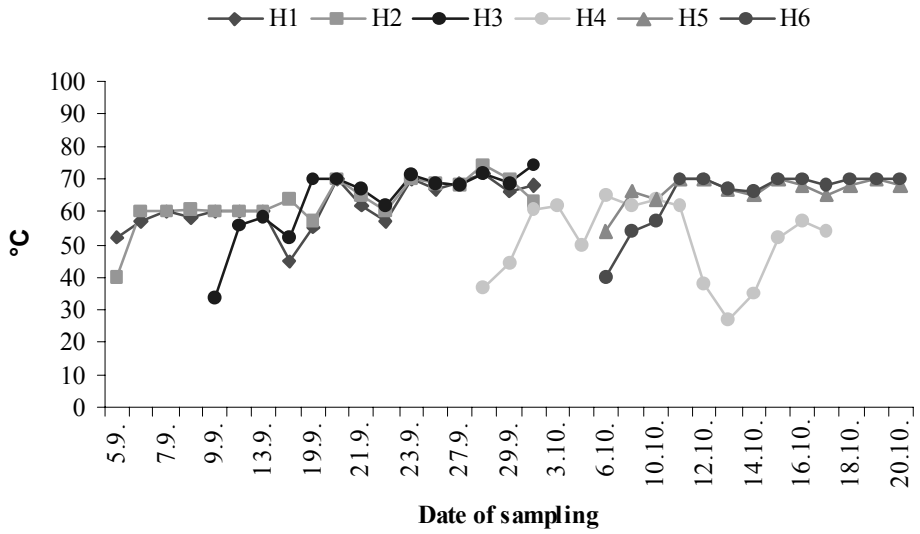

Figure 1. Pile temperature changes during composting

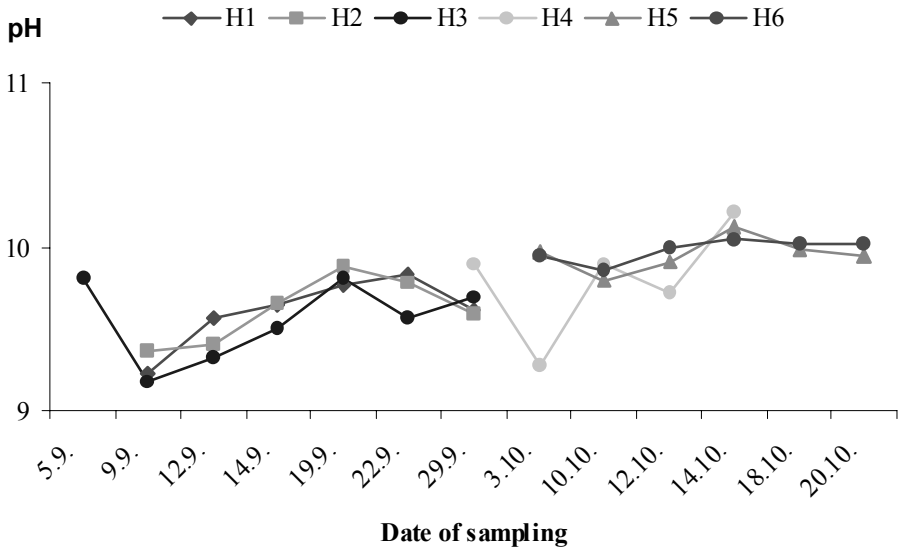

Figure 2. Changes in pH of organic material during composting

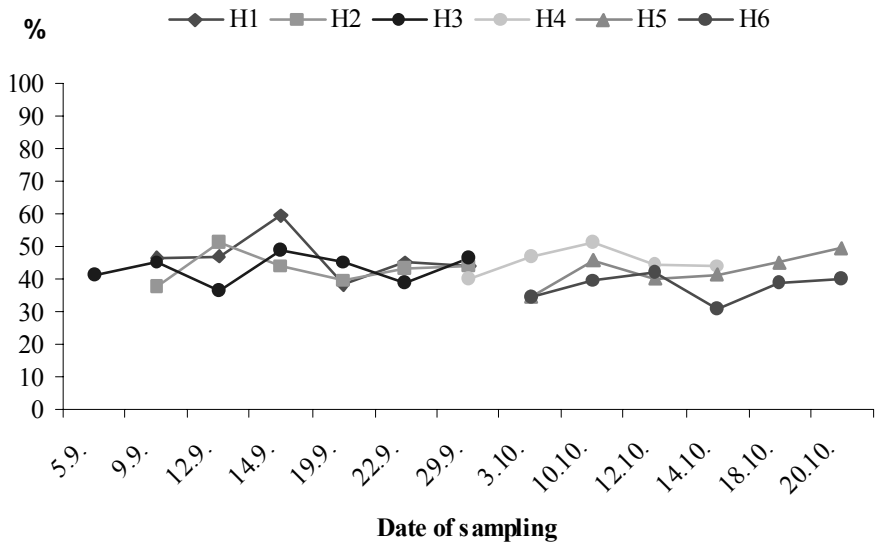

Figure 3. Changes in DM of organic material during composting

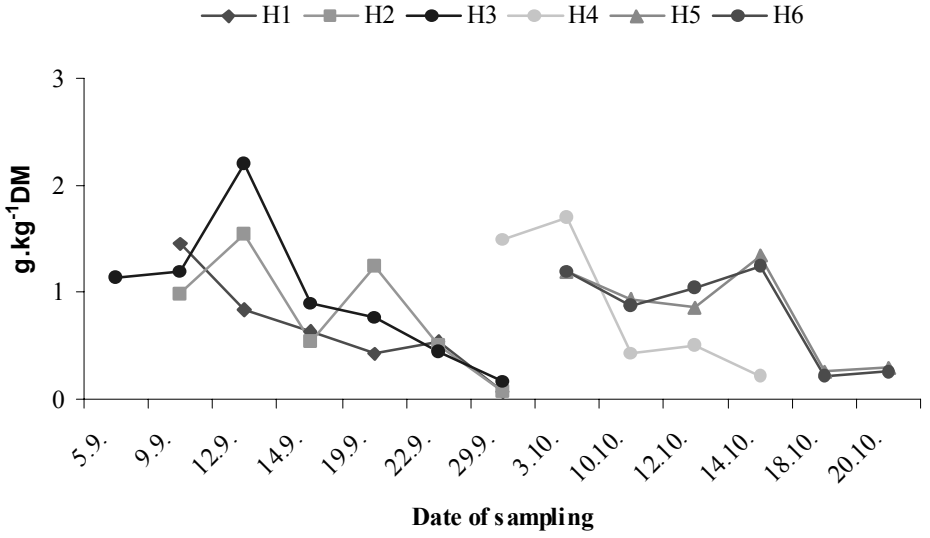

Figure 4. Changes in NH_4^+ content of organic material during composting

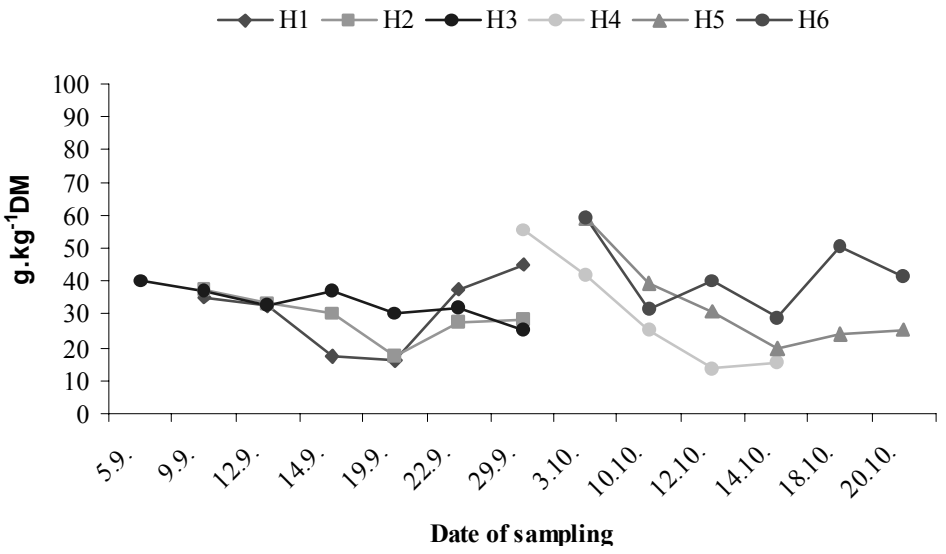

Figure 5. Changes in N_t content of organic material during composting

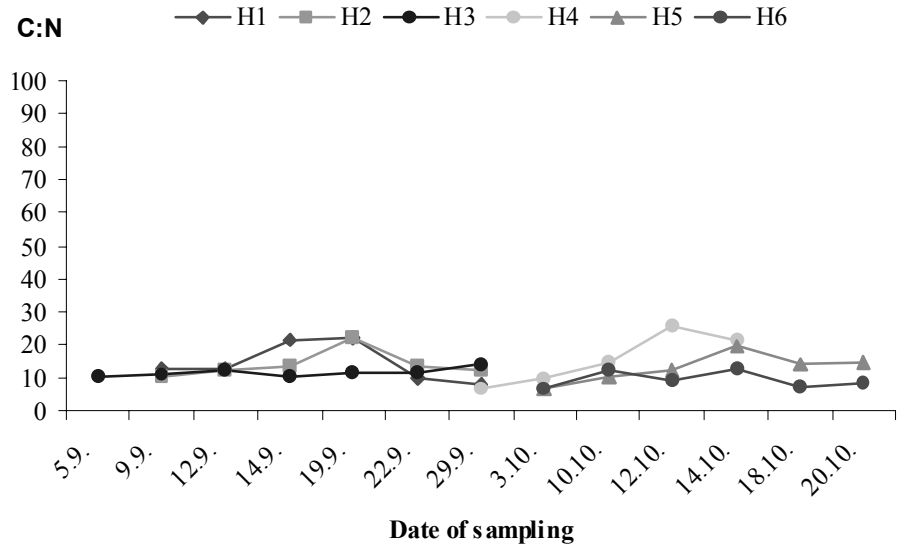

Figure 6. Changes in C:N ratio of organic material during composting

BACTERIOLOGICAL AND PARASITOLOGICAL RISKS ASSOCIATED WITH AGRICULTURAL WASTEWATERS AND SEWAGE SUBJECTED TO BIOLOGICAL TREATMENT

Sasáková, N.[1], Juriš, P.[2], Papajová, I.[2], Vargová, M.[1], Venglovský, J.[1], Ondrašovičová, O.[1], Ondrašovič, M.[1]

[1] *University of Veterinary Medicine, Komenského 73, 041 81 Košice;* [2] *Parasitological Institute of the Slovak Academy of Sciences, Hlinkova 3, 040 01 Košice; E-mail: nada.sasakova@post.sk*

SUMMARY

The aim of our study was to investigate the bacteriological and parasitological risk associated with the products of aerobic treatment of pig slurry and municipal sewage. We focused on the quality of effluent and on sludge/pig slurry solids from two wastewater treatment plants (pig slurry WWTP-1; municipal wastewater WWTP-2). Efficiency of removal of selected bacteria ranged between 79.8 and 97.9%. No helminth eggs were found in effluents. One sample of sludge (out of 40) contained 1 egg of Ascaris spp. and another 1 one egg of Trichuris spp.

Keywords: helminth eggs, bacteria, wastewater treatment, pig slurry, sewage sludge

INTRODUCTION

Agricultural use of organic waste materials is an option favourable from the economical point of view but there are certain aspects that have to be respected with regard to environmental protection and the necessity to protect humans, animals and plants from undesired infections (Papajová *et al*., 2002).

The requirement for spreading diseases by raw and processed wastes is that the material must become infected with the causative organisms, which must survive treatment or storage, remain capable of causing disease and survive in the material until a human or animal host is encountered. The type of pathogens most commonly found in sewage and sewage sludge depends on the state of health of the population, as well as the presence of hospitals, meat processing plants and abbatoirs in the area (Bruce and Davis, 1983). Sewage sludge may contain a large variety of bacterial and viral pathogens including *Salmonella* spp., *Shigella* spp., *Yersinia* spp., and enteroviruses as well as eggs of parasites such as *Ascaris lumbricoides* and oocysts *Cryptosporidium* spp. and *Giardia* spp. (Straub *et al*., 1993).

With regard to disposal of pig excrements, bacteria such as *Salmonella* spp., *Escherichia coli*, *Mycobacterium* spp., *Enterococcus* spp., *Streptococcus* spp., *Staphylococcus* spp. pose a potential threat to animal and human health. In addition to that protozoa (*Isospora* spp., *Balantidium coli*) and eggs or larvae of enteronematodes (*Ascaris suum*, *Oesophagostomum* spp., *Trichuris suis*) are also found in pig faeces. *Ascaris* eggs and coccidial oocysts are hygienically the most hazardous, primarily for their high resistance in the environment (Novák *et al.*, 1998; Dubinský, Juriš and Moncol, 2000).

MATERIAL AND METHODS

In the first stage of our study, we examined samples from waste-water treatment plant (WWTP-1) treating approx. 500 $m^3.d^{-1}$ of pig slurry and 320 $m^3.d^{-1}$ of village sewage. The solid portion of raw wastewater is separated on vibrating sieves before biological treatment and is stabilised later by simple storage for different period of time before application to soil. Samples for chemical and bacteriological examination were taken in monthly intervals. Parasite eggs and oocysts were determined in individual stages of the treatment (influent, effluent, solid fraction).
In the second stage, we investigated samples from wastewater treatment plant (WWTP-2) treating 1300 $l.s^{-1}$ of municipal wastewater. The biological stage in this plant is aerobic and the sewage sludge produced is subjected to anaerobic and aerobic treatment and dewatering.. Samples for chemical and bacteriological examination were taken in monthly intervals for one year and parasitological examination for the presence of helminth eggs and oocysts was performed twice during this period.

Of chemical parameters determined in the study we include only pH as one of the most important factors affecting survival of micro-organisms. Additional results of chemical examinations were reported elsewhere (Venglovský *et al*., 2005).

Bacteriological examination consisted of determination of plate counts of mesophilic, coliform and faecal coliform bacteria (STN 83 0531-4 and STN-ISO 9308-2) on solid cultivation media (Endo agar, Imuna, Slovakia) and faecal streptococci in the municipal sludges (STN-EN ISO 7899-2) on Slanetz-Bartley agar (Biomark, India).

Parasitological examination of solid samples was carried out by the method of Kazacos (1983). The helminth eggs from liquid samples (influent and effluent) were isolated by a sedimentation-floatation method of Cherepanov (1982), which is a modification of the method of Romanenko (1968) using saturated sacharose solution of specific gravity 1.30.

RESULTS

The results of bacteriological and parasitological analysis of samples from WWTP-1 are presented in Tab. 1 and 2. Mean plate counts of mesophilic bacteria ranged between $9.8x10^6$ and $9.2x10^8$ $CFU.ml^{-1}$, of coliform bacteria between $1.0x10^5$ and $8.9x10^8$ $CFU.ml^{-1}$ and of faecal coliform bacteria between $1.0x10^5$ and $8.3x10^7$ $CFU.ml^{-1}$. The numbers of selected groups of bacteria (mesophilic, coliform, faecal coliform) in the influent and effluent from WWTP-1 showed that mean efficiency of removal reached 95.7% for mesophilic bacteria, 86.5% for coliforms and 91.4% for faecal coliform bacteria. This resulted from the decrease in plate counts by approximately two orders of magnitude in most samplings and corresponded to the technology used. Better efficiency was reached in the warmer period (May – October) as the flocculation of activated sludge may be supported by higher temperatures. The plate counts in the solid fraction ($9.4x10^4$ – $3.8.10^9$ $CFU.ml^{-1}$) indicate high population of this substrate with the bacteria of interest. Parasitological examination showed that no helminth eggs were found in effluents from both plants despite their presence in the influent.

Bacteriological examination of samples from WWTP-2 provided different results (Tab. 3). The plate counts of investigated bacteria in the influent were lower due to considerable dilution. Mean plate counts of mesophilic bacteria ranged between $1.4x10^4$ and $4.5x10^5$ $CFU.ml^{-1}$, of coliform bacteria between $6.5x10^4$ and $3.3x10^6$ $CFU.ml^{-1}$ and of faecal coliform bacteria between

$4.1x10^4$ and $7.4x10^5$ CFU.ml^{-1}. The mean efficiency of removal reached 97.9% for mesophilic bacteria, 96.6% for coliforms and 79.8% for faecal coliform bacteria.

None of the samples (n = 10) of influent, effluent and activated sludge allowed us to recover helminth eggs. One of the factors may be the considerable dilution of human faeces in these waste-waters. Out of 40 samples of sludge only two samples were positive, one contained one egg of *Ascaris* spp. and the other one egg of *Trichuris* spp.

DISCUSSION

The epidemiological risk arising from the use of excrements and sludges is related to presence of pathogens in these substrates and their potential survival sometimes for a remarkable period of time. The subsequent direct and indirect transmission of zoonotic agents to farm animals is generally regarded as the most relevant risk factor of agricultural utilization of untreated or insufficiently treated sludge and wastes of animal origin (Juriš *et al*., 2000). Effluents from WWTP are discharged into surface waters where they increase the counts of coliform and faecal coliform bacteria and faecal streptococci. Evaluation of quality of surface waters in Slovakia shows that particularly due to microbiological parameters many rivers belong to lower quality classes (SAŽP, 2004).

The organisms used to monitor the effectiveness of sanitation treatment of organic wastes were *E. coli*, faecal streptococci and *Salmonella* spp. According to Hays *et al*. (1977) considerable number of bacteria and viruses entering the WWTP is devitalised by the treatment but endoparasite developmental stages may remain viable. With regard to their relatively high specific weight they tend to sediment and concentrate in the solid fraction together with undissolved substances and in this manner may be returned into the environment (U. S. Environmental Protection Agency, 1992). While the pathogenic viruses and bacteria may survive in the environment for hours or days, protozoan cysts remain viable for months and eggs of helminths even for years (Sobsey and Shields, 1987) because of the presence of stabilising proteins, lipids and chitin in the wall of nematode eggs (Bruňanská, 1989; Eckert, 2000). In their fully-developed, second-stage larval form, eggs of *Ascaris* spp. are highly resistant and have been frequently used as indicator organisms for water and sewage treatment processes. They may also be a good indicator for the effectiveness of composting to reduce parasites (Mara and Cairncross, 1999).

Our results showed that the efficiency of removal of selected groups of bacteria in both treatment plants corresponded to the technology used. In general, the plate counts decreased by one to two orders of magnitude. Better results were reached in the summer season which can be associated with better flocculation of the activated sludge and therefore also higher entrapment of bacteria in the sludge flocks.

Results of parasitological analysis showed that eggs of several helminths were present in the influent to WWTP-1 while all effluent samples were negative. Examination of the solid fraction indicated that considerable number of them passed to the solid fraction and therefore this substrate requires further processing before application to agricultural land. This indicates that there is a need for additional treatment of this material especially because it is almost exclusively used for agricultural purposes. Composting may be recommended as a most suitable way of treatment as it inactivates most of the agents, provided that temperatures above 55^{o}C are maintained for sufficient period of time. However, some authors reported that resistant organisms such as *Clostridium perfringens*, *C. botulinum* and the cysts and eggs of protozoan and helminth parasites may survive. There is also a danger that *E. coli* and *Salmonella* spp. may grow in the final

compost if the process has been inefficient and the organic matter remains poorly stabilised. According to Day and Shaw (2000) the temperature of 55°C is sufficient to devitalise *Ascaris lumbricoides* in 60 min and *Entamoeba histolytica* cysts in 1 sec. Thermophilic stabilisation (48.5°C) was sufficient to destroy eggs of *A. suum* in the study by Plachá *et al.* (2002). Burge (1983) observed that 10-fold reduction in *Ascaris* spp. ova was reached within 1.3 min at 60°C. All the above data indicate that composting at temperatures above 55°C for at least 3 days should be sufficient to eliminate or at least minimise the problem.

The samples of influent and effluent from WWTP-2 were negative at helminthological examinations. Of the 40 samples of sludge from this plant only two were positive. It is evident that the current sludge treatment cannot guarantee devitalisation of helminth stages and for this reason liming of sludge and its storage at pH higher than 12 for at least 3 months is recommended before its application to the soil.

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Table 1. Plate counts of selected groups of bacteria in the influent and effluent and the solid fraction from WWTP-1

	Influent (CFU.ml^{-1})	**Effluent (CFU.ml^{-1})**	**Efficiency (%)**	**Solid fraction (CFU.kg^{-1})**
Mesophilic bacteria	$9.8x10^6$–$9.2x10^8$	$5.2x10^4$–$1.2x10^7$	95.7	$3.1x10^7$–$3.8x10^9$
Coliform bacteria	$1.0x10^5$–$8.9x10^8$	$2.3x10^3$–$4.1x10^6$	86.5	$3.5x10^5$–$1.6x10^8$
Faecal coliform bact	$1.0x10^5$–$8.3x10^7$	$4.3x10^3$–$6.3x10^5$	91.4	$9.4x10^4$–$2.4x10^6$

Table 2. Parasitological examination of samples from WWTP-1

	Influent (eggs.1000 ml^{-1})	**Effluent (eggs.1000 ml^{-1})**	**Solid fraction (eggs.100g^{-1})**
A. suum	28–29	0	12–35
***Oesophagostomum* spp.**	5–19	0	2–6
***Trichuris* spp.**	1–3	0	1–2
***Hymenolepis* spp.**	0–5	0	0
***Isospora* spp.**	0 – 9*	0*	0*
***Eimeria* spp.**	6 – 34*	0*	2 – 12*

* – oocysts

Table 3. Plate counts of selected groups of bacteria in the influent, effluent and sludge from WWTP-2

	Influent (CFU.ml^{-1})	**Effluent (CFU.ml^{-1})**	**Efficiency (%)**	**Sludge (CFU.ml^{-1})**
Mesophilic bacteria	$1.4x10^4$–$4.5x10^5$	$1.0x10^3$–$5.7x10^4$	97.9	$1.5x10^6$–$8.9x10^7$
Coliform bacteria	$6.5x10^4$–$3.3x10^6$	$1.0x10^2$–$4.4x10^3$	96.6	$2.2.10^6$–$1.3x10^8$
Faecal coliform bacteria	$4.1x10^4$–$7.4x10^5$	$1.1x10^3$–$2.8x10^5$	79.8	$8.6.10^5$–$9.4x10^7$

ELIMINATION OF *ASCARIS SUUM* EGGS DURING COMPOSTING OF ORGANIC WASTE IN THE KNEER CONTAINER TECHNOLOGY

Szala, B. and Paluszak, Z.

Department of Microbiology, University of Technology and Life Sciences. Bernardyńska 6/8, 85-029 Bydgoszcz, Poland; mikro@utp.bydgoszcz.pl

SUMMARY

Inactivation of *Ascaris suum* eggs is very important aspect of hygienization during composting process. The elimination of *A. suum* eggs was evaluated during composted waste from municipal green areas mixed with sewage sludge using the Kneer container technology. Carriers containing eggs were introduced into the material in a container and then into a windrow. The results of the study indicated a high efficiency of composting in uppers layers of biomass. The theoretical time of their survival ranges from 16 to 20 days. Inactivation of eggs in the bottom layer proceeded very slowly. Daily decrease in number of alive eggs ranges from 0.11% to 0.94%.

Keywords: *Ascaris suum*, sewage sludge, composting

INTRODUCTION

Composting is one of commonly used methods of organic waste utilization [7]. Besides the traditional way of composting in piles, the in-vessel systems, where there is a considerably higher possibility of the efficient process handling arouses a growing interest. Their application will permit us to reduce in gas emission, shorten particular stages of the process, require less space and it ensures a higher level of environmental biosafety [2,6,9]. In Poland, one of numerous applied technologies is the container Kneer system technology, which is used for municipal waste utilization. Due to frequent addition of sewage sludge to composted materials, there is a high risk of biomass contamination with pathogenic microorganisms and enteric parasite eggs. The inactivation of eggs of the genus *Ascaris*, frequently present in sewage sludge, is an essential aspect of hygienization during the composting process [1,3,4,5], as helminth eggs are characterized by a high resistance to unfavourable environmental conditions, including high temperatures [8,10,11]. The aim of the study was to estimate the effectiveness of *Ascaris suum* eggs elimination during the composting process using the container technology of Kneer type.

MATERIAL AND METHODS

The research was conducted in a composting plant of waste from municipal green areas and sewage sludge working in the container technology of the system Kneer. Properly fragmented and mixed material was placed in containers, where a stage of intensive composting proceeded for approximately 14 days. Then the biomass was removed from the containers and formed in a windrow. In order to provide a proper aeration, the windrow was turned mechanically every two weeks. The stage of the biomass maturation lasted about 4–6 weeks.

The hygienization level of waste subjected to composting was tested on the basis of the inactivation of the enteric parasite *Ascaris suum* eggs inactivation. Eggs were isolated from prepared uteruses of sexually mature females, which were squeezed with a glass rod into a solution of saline on a Petri dish. Next, 1 ml of their suspension was introduced into perlon bags of 28μm pore diameter. Prepared carriers were placed in the composted material in a container at the top, medium and bottom layer of the biomass. During the stage of intensive composting the carriers were removed several times from each layer and subject to analyses. After forming the windrow, part of the carriers was transferred into it from the container, and additional carriers were introduced, containing the eggs of the helminth *Ascaris suum*. Every several days the carriers were removed, cut out and placed in sterile Petri dishes. The bags were poured with water and incubated for 30 days at a temperature of 28ºC. The dishes were opened in order to provide oxygen and filled up with water. After the incubation, 300 eggs were observed under the microscope, and the percentage of invasive eggs, containing live larvae, was calculated. The control was the eggs incubated directly after taking from the fragments of *Ascaris suum* uteruses. The results obtained were subjected to statistical analysis using the program Statistica. Regression lines were drawn and were a basis for the calculation of the theoretic time needed for the inactivation of *Ascaris suum* eggs.

RESULTS AND DISCUSSION

The results of analyses showing the elimination of *Ascaris suum* eggs were presented in Table 1 and in Figures 1 and 2.

A considerably similar course of the hygienization process was observed in the investigations conducted in the two containers. In the carriers in container I, *Ascaris suum* eggs died fastest in the top layer of the biomass. After 13 days, microbiological analyses showed the presence of 19% of invasive eggs here, and after 5 successive days no more eggs able to develop were found (Fig.1). In the medium part the inactivation proceeded in a similar way. The calculated time of the full elimination of parasite eggs amounted to 20 days (Tab.1). In the bottom layer, however, egg inactivation proceeded very slowly. After 4 days, the percentage of viable eggs decreased slightly to 95% and it remained at this level until the 29th day, when the analyses of the content of the carriers, transferred earlier from a container to the windrow, were made for the last time (Fig.1).

In container II, after 18 days of the process no viable eggs were present in the carriers from the top and medium layers (Fig.1). Yet in the bottom layer, the theoretical time of eggs survival was very long and amounted to 101 days. Their daily decrease calculated on the basis of regression line equations was slight – 0.94% (Tab.1).

In new carriers additionally introduced into the windrow the elimination of *Ascaris suum* eggs in the upper and central part preceded similarly. It has been indicated that after 5 days from placing the carriers in the windrow *Ascaris suum* eggs did not remain maintain the ability to further development (Fig.2). Yet in the bottom layer the number reduction rate was very slow and amounted to 0.46% /day. Calculated statistically, the time needed for their full inactivation was 205 days (Tab.1).

The results obtained confirm a high hygienization efficiency of composting process in the upper layers of biomass. The inactivation did not occur during the stage of intensive composting in containers, which was connected with too low temperature generated during the process (Fig.3). After forming the windrow, however, mixing the material and its better aeration resulted in temperature growth. At the same time, no viable *A. suum* eggs were recorded (Fig.4).

Numerous authors' report that in thermophilic conditions occurs the full elimination of enteric parasite eggs [8]. Gantzer [3] claims that a temperature above 45ºC is sufficient for obtaining a hygienic product.

Attention should be given to the bottom layer of the composted biomass, where both in the containers and in the windrow the full inactivation of the eggs was not achieved. The phenomena was connected with the lack of the thermophilic stage in this layer of the composted material. Strauch [12] reported that composting ensures hygienization of the material on condition that all biomass is exposed to a sufficiently high temperature (55ºC for 14 days). Also Gaspard [4] indicated that *Ascaris* eggs maintain their ability to growth after 30 days of composting, if an even temperature distribution over all the biomass is not ensured.

Using the compost where enteric parasite eggs were not totally destroyed in agriculture may result in soil contamination. Studies by Strauch [13] prove that *A. suum* eggs that get do soil will not lose their viability even for 14 years. Therefore, particular emphasis should be put on the full elimination of *Ascaris* eggs in all parts of composted material, by means of the proper handling and control of the process of sewage sludge utilization.

Table 1. The dynamics of *Ascaris suum* eggs elimination in the composted material

Location carriers	Layers of biomass	Regression equations	r^2 (%)	Survival of eggs (days)
container I	top	y = –5.78x + 99.73	98.01	17
	medium	y = –5.09x + 106.54	88.36	20
	bottom	y = –0.11x + 97.13	32.49	883
container II	top	y = –5.22x + 84.86	84.64	16
	medium	y = –5.89x + 101.62	92.16	17
	bottom	y = –0.94x + 94.89	88.36	101
windrow	top	–	–	nd (after 5 days)
	medium	–	–	nd (after 5 days)
	bottom	y = –0.46x + 94.39	73.96	205

nd – no occurrence detected of viable eggs

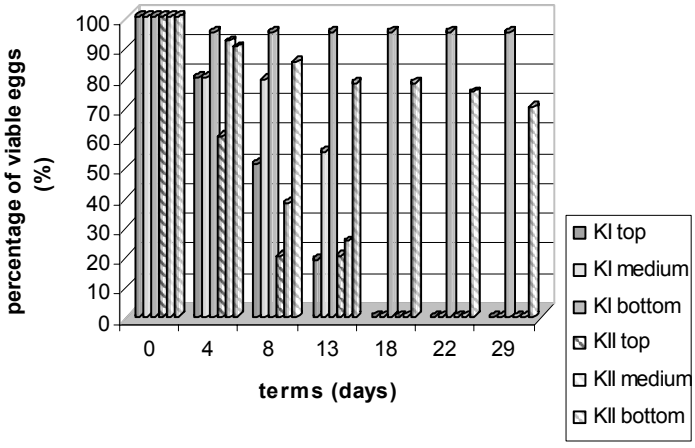

Figure 1. Inactivation of *Ascaris suum* eggs during composting process of biomass in the container I (K I) and container II (K II)

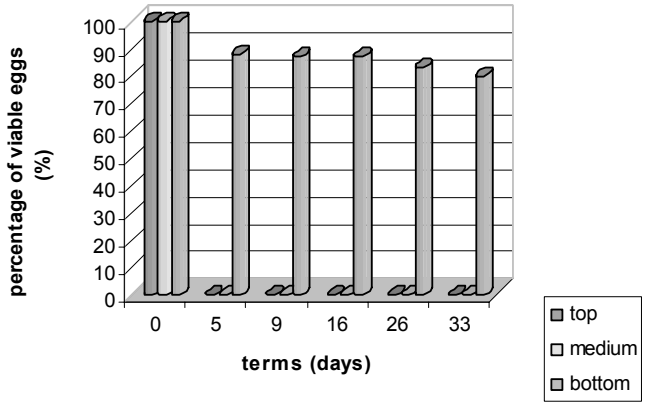

Figure 2. Inactivation of *Ascaris suum* eggs during composting process of biomass in the windrow

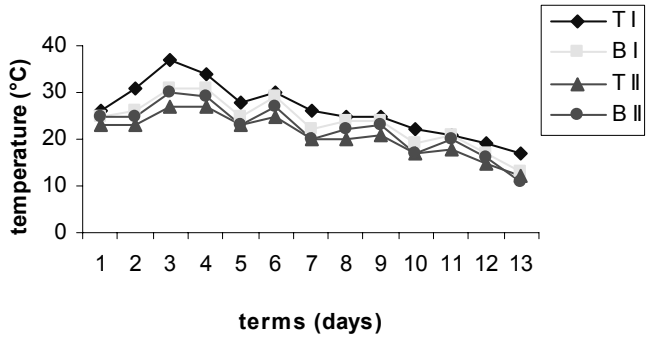

Figure 3. Temperature distribution in the composted biomass in the container I and container II, in the top (T) and bottom (B) layer

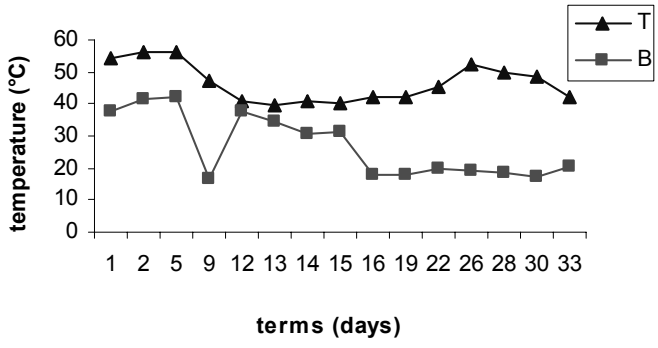

Figure 4. Temperature distribution in the composted biomass in the windrow, in the top (T) and bottom (B) layer

CONCLUSIONS

1. The research showed a high effectiveness of the composting process in the inactivation of *Ascaris suum* eggs using the container technology of the type Kneer in the top and medium layer of the biomass.
2. The bottom layer of the composted material makes the hazard area, due to the slow rate of eggs elimination.
3. The focus should be on improving the aeration conditions, thereby obtaining a properly high temperature in all parts of the composted material.
4. A simple and effective method for research used in the experiments allows having a direct control over the hygienization process of the composted material.

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NOISE IN THE ANIMAL HOUSING ENVIRONMENT

Venglovský, J. [1], Sasáková, N. [1], Vargová, M. [1], Ondrašovičová, O. [1], Onrašovičová, S. [1], Hromada, R. [1], Vučemilo, M.[2] and Tofant, A.[2]

[1] *University of Veterinary Medicine Kosice, Komenskeho 73, 041 81 Kosice, The Slovak Republic;*
[2] *Faculty of Veterinary Medicine University of Zagreb, Croatia; E-mail: jan@venglovsky.com*

SUMMARY

Noise produced in intensive animal rearing by ventilation system, feeding and excrement removal lines and by animals themselves is a potential stressor and affects not only animals but also the tending personnel. High sensitivity to noise levels has been observed in pigs with some potential impact on their behaviour. In our study we measured exposure of pigs to noise in 3 houses for three categories of pigs, farrowing house, house for weanlings and house for sows before mating and after confirmation of gravidity. Although our measurements failed to identify high exposure of pigs to noise, this issue should be monitored further to avoid unnecessary stress in this very sensitive species of animals.

Keywords: noise, pig housing

INTRODUCTION

With the widespread use of intensive rearing systems, animals are increasingly exposed to several stressful situations engendered by farm management practices. Handling of animals, confined housing conditions or social stress in group housing are strong stressors throughout the life of farm animals causing acute or chronic activation of the hypothalamo-pituitary-adrenocortical (HPA) axis and the sympatho-adrenomedullary (SAM) system (Otten et al., 2004).

Noise is a potential environmental stressor and has also been identified as an aversive stimulus during animal housing. Animals are exposed to greater noise by the mechanization of many husbandry procedures. The noise produced in animal production affects the tending personnel and veterinarians and may even lead to damaged hearing (Jackson 2002). The damage to hearing is insidious in its nature because it occurs over some time and when the levels are sufficiently high this damage can be irreversible. The damage occurs when the hair like cells (cilia) that receive the sound waves are repeatedly or very violently flattened. Initially, given enough quiet time for regeneration, the damage may be reversible. Because of that the maximum noise level allowable over an eight hour period is 85 dB. Longer exposure to higher levels may result in damage.

There are contrasting reports regarding the influence noise may have on the physiological, behavioural and productive traits of animals, especially because response to sound stimulation are species-specific and largely depend on the nature, loudness and familiarness of the noise.

The exposure of farm animals to noise has been identified as a potential stressor not only in housing (Talling et al., 1998a; Schäffer et al., 2001) but also during the transport and at the abattoir (Geverink et al., 1998). Noise experienced during housing of farm animals can be short-term and acute (e.g. screaming before feeding times) or uniform and chronic or chronic intermittent (e.g. basal sound levels caused by crowded animals, mechanical ventilation). Average

sound pressure levels ranging between 69 and 78 dB were recorded in fattening units of pig farms, between 88 and 96 dB during transport and between 85 and 97 dB at the abattoir (Talling et al., 1998a). Behaviour of piglets and sows during suckling in relation to sound levels were investigated by Bo Algers et al. (1985). The external noise changed the vocalisation feeding pattern so that the noise-exposed piglets gained less milk and their weight gains were affected.

Our study was aimed at measurement of noise level on a pig farm in houses for different categories of pigs.

MATERIAL AND METHODS

Measurements were carried out on a pig farm in the house for weanlings, farrowing house and house for sows before mating and farrowing sows. In the farrowing house the conventional way of housing with some bedding was used with area of pens divided to the part only for sow and that available only to piglets which prevents sows to overlay the piglets. The weaned piglets were housed in group pens with automatic *ad libitum* feeding and partly solid, partly perforated floor. Sows before mating and confirmation of gravidity were housed individually and gravid sows were on deep litter. They were both in one house.

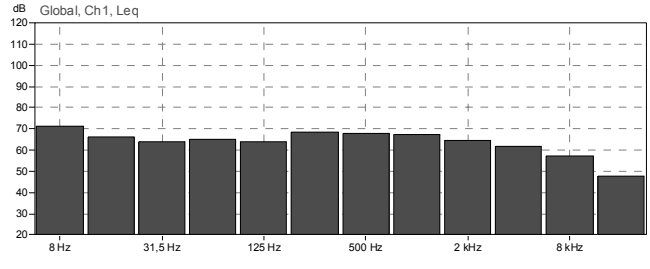
The measurements were carried out with an integrated noise measurement apparatus NORSONIC 118, accuracy class 1, with 1/1 frequency analysis.

RESULTS AND DISCUSSION

Results obtained in our study are presented in Fig.1. – 3. and Tables 1 – 3.

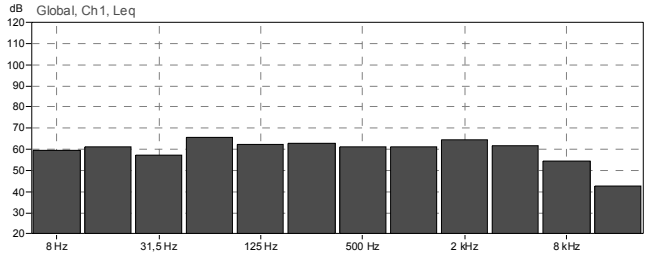
Weanlings (from 5–7 to 30–35 kg b.w.)

	Leq (dB)	Lpeak (dB)
A	**72.1 dB**	**107.3 dB**
C	74.8 dB	107.0 dB
FRQ		
8 Hz	71.0 dB	
16 Hz	66.2 dB	
31.5 Hz	64.1 dB	
63 Hz	65.0 dB	
125 Hz	63.8 dB	
250 Hz	68.3 dB	
500 Hz	67.9 dB	
1 kHz	67.6 dB	
2 kHz	64.7 dB	
4 kHz	61.9 dB	
8 kHz	57.5 dB	
16 kHz	47.7 dB	



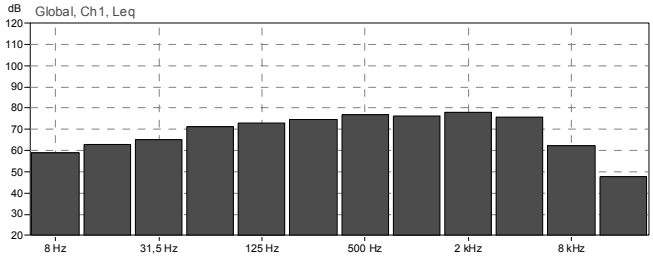
Farrowing house

	Leq (dB)	Lpeak (dB)
A	**69.1 dB**	**101.5 dB**
C	71.5 dB	100.7 dB
FRQ		
8 Hz	59.4 dB	
16 Hz	61.0 dB	
31.5 Hz	57.5 dB	
63 Hz	65.9 dB	
125 Hz	62.4 dB	
250 Hz	62.9 dB	
500 Hz	61.0 dB	
1 kHz	61.4 dB	
2 kHz	64.3 dB	
4 kHz	61.7 dB	
8 kHz	54.5 dB	
16 kHz	42.7 dB	



Sows before mating and gravid sows

	Leq (dB)	Lpeak (dB)
A	**83.1 dB**	**113.8 dB**
C	83.9 dB	**114.9 dB**
FRQ		
8 Hz	59.2 dB	
16 Hz	62.7 dB	
31,5 Hz	65.2 dB	
63 Hz	71.3 dB	
125 Hz	72.9 dB	
250 Hz	74.5 dB	
500 Hz	76.8 dB	
1 kHz	76.4 dB	
2 kHz	78.0 dB	
4 kHz	75.5 dB	
8 kHz	62.2 dB	
16 kHz	47.5 dB	



Effects of acute and chronic noise exposure on the behaviour as well as on the neuroendocrine and immune system were observed in different species (Segal et al., 1989; Raaij et al., 1996).Very little information is available about acute or chronic noise effects on pigs. Acute sound exposure was found to increase active behaviour and heart rate (Talling et al., 1998b). A single and short-term noise exposure of pigs at 120 dB was found to increase plasma glucocorticoid concentrations, but had no effect on plasma catecholamines (Kemper et al., 1976).

The sources of harmful noise in animal production are various: feeding 104–115 dB, mating 94–115 dB, high-pressure cleaning 105 dB, feed mixing 88–93 dB. However, these values are only orientational and may differ according to the technologies used. There are respective regulations which set the minimum requirements on protection of herds for individual categories

of animals. For pigs, which are very sensitive to changes in noise levels, these requirements are specified by the Statutory Order of SR No. 325/2003 that amends and supplements the Statutory Order of SR No. 735/2002 of the Civil Code specifying minimum standards for protection of pigs. In the part of a building where pigs are reared the noise level must not exceed 85 dB and there are also limits on background or sudden noise.

Different levels of noise were observed in pigs in relation to the type of ventilation. The sound level measured in mechanically ventilated pig buildings was 73 db but naturally ventilated buildings were on average 10 dB quieter. The frequency of sound on farms is also important and ranges between 20 to 6 300 Hz.

Our results did not indicate high exposure to noise of pigs in different houses for individual categories. However, with regard to the fact that even short-lasting but intensive noise can have harmful effect not only on animals but also on personnel this issue requires further monitoring and attention.

CONCLUSION

The noise issue in agriculture has recently attracted considerable attention with regard to both animal well-being and working conditions of animal tenders. High sensitivity to noise levels has been observed in pigs with some potential impact on their behaviour. Some sources of noise (ventilation system) result in almost constant exposure while others can produce short-lasting but intensive noise (feeding and manure removal lines). Although our measurements failed to identify high exposure to noise of pigs, this issue should be monitored further to avoid unnecessary stress in this very sensitive species of animals.

ACKNOWLEDGEMENT

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G –
ANIMAL TRANSPORT, EUTHANASIA AND SLAUGHTER – CAN GOOD WELFARE BE PROVIDED?

ORAL PRESENTATIONS

MEASUREMENT OF THERMAL STRESS IN SLAUGHTER CATTLE DURING LONG ROAD TRANSPORT

Hartung, J.[1], Marahrens, M.[2] and Brüser-Pieper, C.[1]

[1] *Institute for Animal Hygiene, Animal Welfare and Behaviour of Farm Animals, University of Veterinary Medicine Hannover,* [2] *Germany; Federal Agriculture Research Institute, Celle, Germany*

SUMMARY

Heat stress is seen as one of the major aversive factors during long distance road transport of cattle entailing poor welfare. The thermal environment of 587 slaughter bulls was continually monitored inside the lorry during 15 trips for 24 h (mean) between July and October from Germany to Italy and blood samples were analyzed for stress indicators. The stress factors analyzed in the blood remained within limits that did not show any detriment or damage to the animals. None of the animals became clinically ill. The risk of heat stress in slaughter cattle on long transports seems to be overemphasized if vehicles and organization are appropriate.

Keywords: cattle, long transport, heat stress, THI, blood constituents

INTRODUCTION

In 2002 about 80 million cattle were kept in the European Union. Most of these animals are transported less than 8 hours and 45 million cattle are transported not longer than 4 hours with about 16 million going to nearby farms and about 29 million to slaughter houses. The number of cattle being transported longer than 8 hours is less than 2% only but attracts most public attention. These animals are usually imported from or exported to third countries (for 2002: about 530,000 cattle imported and about 300,000 exported) (Schons 2003). One main concern associated with such long transports is heat stress the animals may suffer during the transport journey.

This paper reports about temperature and humidity measurements on lorries during long transports of slaughter bulls from the north of Germany to the Mediterranean port of Trient in Italy in order to address the thermal stress experienced by the animals (Brüser-Pieper 2006). The journey times were for up to 27 hours. Blood samples were taken from a number of indicator animals on each transport to analyze certain blood constituents which may be associated with stress reactions.

MATERIALS AND METHODS

The thermal environment of 587 slaughter bulls was continually monitored during fifteen trips between July and October 2002, and described according to the itinerary traveled. Temperature, relative air humidity and air movement were thereby measured in 4 of the 5 bays of the transport vehicles. Direct comparisons to the outside climate were made. On each transport, blood samples were taken from 190 indicator animals 12 hours before loading, directly before loading, at the end of the transport, directly after unloading and 24 hours after the end of the rest period in the port of Trieste. The following parameters were determined from these blood samples and compared to clinical reference ranges: cortisol, creatinine kinase, glucose, non-esterised fatty acids, β-hydroxybutyrase, total protein, sodium, potassium, hematocrit, magnesium, triiodthyronine (T3), and thyroxine (T4). The results were interpreted in light of the environmental conditions of the animals, so that it was possible to draw a conclusion on the effect of the observed thermal stress to the animals' health and well-being on transports of up to 27 hours.

RESULTS

Figure 1 shows the course of the mean temperatures measured over about 24 h inside the vehicles during 15 transports of slaughter bulls from the north of Germany to Triest in Italy between July and October 2002.

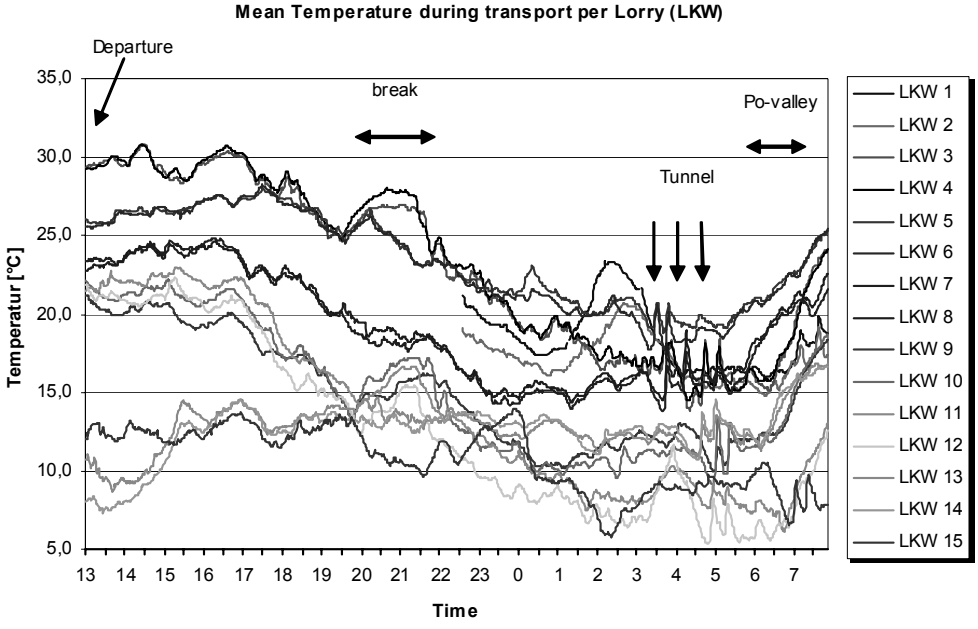

Figure 1. Mean temperatures during 15 transports of slaughter bulls from the north of Germany to Triest in Italy during 24 h

The temperature inside the lorry depends very much on the outside temperature and the waiting time when the lorry is loaded. During the hot season 30 °C are reached before departure. During the journey the inside temperature decreases with the increasing natural ventilation. Temperatures are falling in the evening and during the night. Breaks and tunnel passages increase temperatures again because of lacking ventilation or increased temperatures inside the tunnel. In the morning the temperatures are increasing with the rising sun in the river Po valley. It is important that the animals are unloaded immediately after arrival at destination in the harbour.

Table 1 gives the corresponding relative humidity of the air inside the lorry as means with minimum and maximum values. When the temperatures are high during driving the relative humidity is low and vice versa, when the lorries are standing fully loaded both temperature and humidity is increasing.

Table 1. Relative humidity of the air inside transport vehicles with slaughter bulls during 13 journeys from the north of Germany to Triest in Italy

LKW/lorry	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15
Minimum	–	–	45,2	46,4	57,3	56,3	41,5	41,0	63,6	54,4	51,6	48,0	57,7	57,9	35,2
Maximum	–	–	88,8	86,7	94,5	92,3	86,8	87,0	86,9	90,2	93,8	92,8	97,7	95,9	84,6
Mean	–	–	**68,7**	**69,5**	**74,4**	**73,1**	**61,9**	**60,9**	**73,9**	**71,9**	**70,6**	**70,9**	**81,2**	**79,3**	**69,4**

Both temperature and humidity can act together as stressors for animal and man. Thom (1959) introduced a thermo-humidity-index (THI) to characterize the combined effect of the environmental climatic conditions (dry temperature, relative humidity, dew point). This concept was adapted for cattle in the Livestock Weather Safety Index (LCI 1970). Table 2 gives the categories from normal (<74) to acute danger of life (>84).

Table 2. Thresholds of the Livestock Weather Safety Index (LWSI) and the basic THI values

THI	< 74 normal
	75–78 threshold of thermoregulation
	79–83 danger
	> 84 acute danger of life

Figure 2 shows the distribution in % of all THI classes in lorries 3–15 between departure and arrival. THI values higher than 78 were observed during two journeys in summer for about 24% and 10% of the journey time. These time spans were, however, composed of different periods at the beginning of the journey after loading, shortly during breaks at day time and again before unloading at destination. It is presently not known what the adaptation capacity of slaughter bulls for increasing heat burdens during transport is. It may be assumed that short periods of increased temperatures are tolerated by the animals. Here more research is needed.

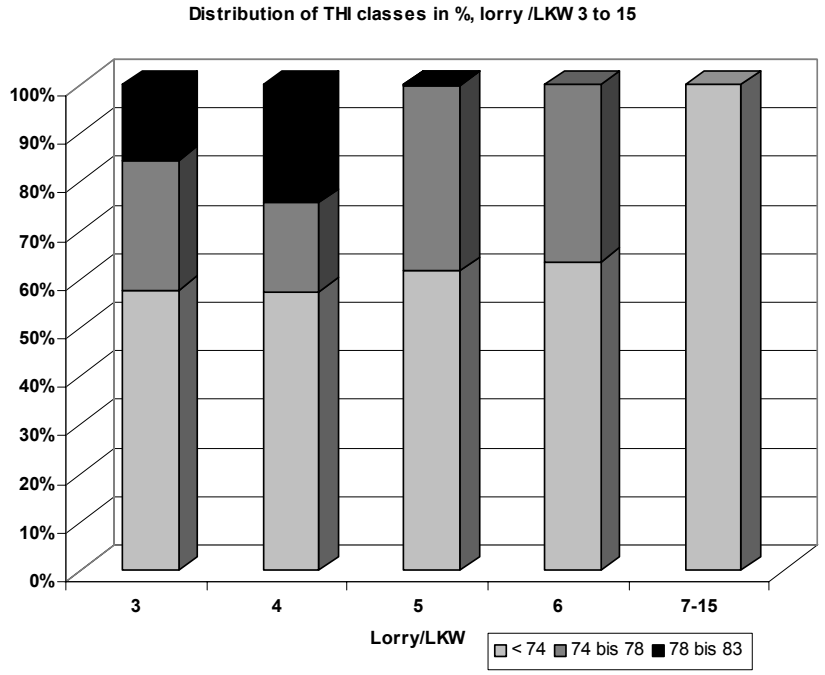

Figure 2. Distribution in % of all THI classes in lorries 3–15 between departure and arrival. Classification according to Livestock Weather Safety Index (LCI 1970)

DISCUSSION

It became clear that the thermal stress experienced by the slaughter bulls was primarily dependent on the season. During four transports in August, average values of 70 THI-units were found in proximity of the animals, whereby maximum values of 78 and 82 units were measured for short periods of time until arrival at the port. The later transports were found to average fewer than 65 THI-units. Air movement through ventilation in the moving vehicle was found to be sufficient to keep the temperature and relative humidity within endurable limits. When the vehicle was not moving however, the air movement fell to low values and resulted in a continuous increase of temperature and relative humidity for the duration of the stop. These increases were more significant if the vehicle stood in direct sunlight and with decreasing winds.

The analysis of the blood samples showed that not all parameters were influenced by the transport. However, clear differences from reference ranges were found before loading in the values for cortisol, glucose, hematocrit and triiodthyronine and mirrored the general stress reactions of the animals to the unknown situation. After arrival at the port and an average of 23.5 hours of transport, the values that had been elevated before transport remained relatively elevated. It was still possible, however, to conclude that the animals got habituated to the transport situation as they progressed. In addition to the previous parameters, the volatile fatty acids were found to be increased after unloading. This was attributed to reduced feed consumption during transport. On arrival, the values for creatinine kinase were found to be increased and continued to increase

strongly during the 24 hour stop at the port. This is attributed to the new grouping of the bulls after transport, which led to constant rank fighting and drastically increased physical activity. There were indications that, particularly in the summer transports, certain animals did not sufficiently access the continuously available water sources.

In general, the magnitude of the increase of the stress factors in the blood samples beyond the reference ranges remained within limits that did not show any detriment or damage to the animals. None of the animals during the 15 transports became clinically ill. There was no indication that the animals' thermoregulatory capacities could have been overwhelmed. It seems that slaughter bull transports of 22 to 27 hours can be accomplished without putting exaggerated strain on the animals. However, the results demonstrate that the animals can be submitted to significant thermal stresses during transports in the summer, especially during stops. Through careful planning and mindful executing of transports, with adequately trained personnel, it is possible to minimize the stress on the animals before critical temperatures are reached. The future records of the position of the vehicles (e.g. by global positioning systems, GPS) may also help to avoid heat stress by early advisory models.

ACKNOWLEDGEMENT

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MODES OF TRANSPORT AND TRANSPORT LORRIES UPON LONG TRANSPORTS OF HORSES, CATTLE, CALVES, ADULT AND YOUNG OSTRICH

Štefančič, I.[1] and Dobeic, M.[2]

[1] Veterinary Administration of the Republic of Slovenia, Animal welfare Section, Partizanska 109, 6210 Sežana, Slovenia, e-mail: i.stefancic@gov.si; [2] Veterinary faculty of Ljubljana, Institute for Environmental and Animal Hygiene with Ethology, Gerbičeva 60, 1000 Ljubljana, University of Ljubljana, Slovenia, e-mail: martin.dobeic@vf.uni-lj.si

SUMMARY

Transportation conditions in the time of animal transports, animal care, properly and technically well equipped transport vehicles are closely connected to administrative rules which can be occur like indicators of animal welfare. One of the most significant welfare deteriorations can be expressed by lack of necessary space (surface and altitude) due to the animal overcrowding and issued exaggerating live body weight to square meter of animal transporting vehicle loading place. In our study we deal with 16.954 animal transport vehicles, 8027 lorries and 8907 railway carriages where 426.079 animals, 248.008 cattle and 161.348 horses for slaughtering, 3088 cattle, 11.053 calf and 310 horses for further breeding and 662 adult ostrich in 12 animal transport lorries as well as 810 young ostrich for breeding in 8 lorries were transported.

Keywords: veterinary medicine, transport, transport lorries, horses, cattle, calves, ostrich

INTRODUCTION

Animal transport is tightly connected to animal production and its goods as well it represents very important economic factor in the national and international traffic (1). Legal rules which concern animal transports and respective ethical norms can be distinguish in the facts that ethical norms do not defeat legislative rules which are taken into consideration up to personal responsibility (2). Positive influences on animal welfare due to transport vehicle equipment can be exposed only if they are in harmonisation with animal breeding technology, animal loading manners and modes of transport of different species of animals, as well as suitable education of drivers and other who deal with animal transports. Due to necessary animal transports, transport organisers, performers and organisations who supervise animal transportation should closely care and consider about animal well being and welfare (2). Among the other investigation of human responsibility to animals is one of most important tasks of veterinary profession (3). It should be considered that animal transport is obligatory and essential part of way to slaughtering plant. Owing to intensive animal production as well as slaughter concentration and animal processing, transport distances in EU prolonged exceedingly. If we pay more attention to obligate measures and preserving animal welfare, animals will be less suffering and more protected against pain, suffering, fear, injuries and death (2, 4). Researching in the fields of animal transport is directed to animal burdening and immunity system weakness (5,3,6,7,8,9). In the present study we want to review different modes of animal transports in periods from 1980 to 1995 and 2003, 2004 by road and railway involving

road lorries and railway carriages as well as influences owing to transports especially due to necessary prescribed space in transport loading places – surfaces and altitudes in comparison to at that time valid national legislation and legislation in EU.

MATERIAL AND METHODS

In investigation the data about transport vehicles and animals transportation were evaluated. Research works were performed on the road and railway border stations in Sežana and Port of Koper, Slovenia. Animals we investigate were transported from third countries to EU and from EU to the third countries. To identify stressful or hazardous steps in transports the study involves:

- 8027 lorries and 8907 railway carriages in the period from year 1980 to 1995 where 248.008 cattle and 161.348 horses for slaughtering were transported,
- 3088 cattle, 11.053 calves and 310 horses for breeding,
- 20 vehicles in the period from year 2003 to 2004 where 662 adult ostrich and 810 young ostrich for breeding were transported.

Studies of transportation influences on animal species and modes of transports were performed. The effects of categories – purposes and modes of transports were observed on:

- horses and cattle for slaughtering horses, cattle, calves and ostrich for further breeding

Through evaluation of transport vehicles we consider:

- length, width, surface, barriers, covering, ventilation, bedding, excrement eliminating, drinking equipment and transport vehicles marking.

Road – animal transport lorries

Surfaces of loading platforms were: 15m², 25m², 35m², 45m² in 55 m².
Lengths: 6,52 m to 12,5 m.
Inside widths: 2,30 m to 2,35 m.
Outside widths: 2,35 m do 2,40 m.
Surface area of most lorry loading places wasn't known that is why we have to measure. Some of lorries were used for cattle and horse transports. Constructions of chassis were different especially on lorries with surfaces from 15m² to 25m² and suitable for transportation of different species of animals, mostly for cattle, horses and in some cases for small cattle and pigs as well. Loading platforms on those lorries can be mechanically lifted by steel wires round the winches. More than 90% of platforms were wooden and consecutive slippery meanwhile in other cases they were made from rip sheet metal, rarely covered with rubber. Lorry sides, so as loading and unloading platforms were made of wood. Platforms were covered with transverse wooden or metal profiles. Ramp slopes vary from 30 to 40º, with no lateral sides and no firm roofs as well as no animal separating barriers. In the winter they were covered by tilts. Transport platforms were covered with hay, sawdust, corn chops, in some cases with hay straw and sand. Lorries were not equipped by drinking equipment and excrement containers. Lorries of larger surfaces 35m², 45m² and 55m² were constructed in two floors for cattle, calves, horses and ostrich transportation. Lorries with transport platforms of 35m² and 45m² were constructed like trailers, and equipped with hydraulic lift devices (transport platforms). Loading platforms were made of rip sheet metal, covered by cca. 2% of hard rubber covering. Both loading places sides were stabile and rigid, combined with doors in every floor, made from metal, with hard metal roofs. Animals were suitable protected to outdoor weather influences. Slots in sides of lorries were attended for adequate ventilation, so as constructed with loading platforms with 30º slopes and horizontal profiles of aluminium. Horses

were not transporting in floors since the may of 1999. We can notice that in the harbour of Koper in years of 1994 and 1995 some lorries for cattle transportation were equipped by barriers. The same can be noticed on lorries for ostrich transportation in years 2004/05.

Railway wagons

Space areas of examined railway wagons were 27m², 33m², 40m², 41m² and 52m². Regular and special two and four axle railway wagons were used for international animal transports. Several construction solutions due to length, load capacity, door position, number and position of ventilation openings were used. Regular wagons were well sheltered against outdoor weather influences. At that time those vehicles can be exceptionally used also for human transports, what we can read in guides and catalogues published from railway wagon transporters. We measure and summarise some measurements of loading loading places:

- length 10,45 m to 20,40 m
- width 2,59 m do 2,66 m.

Doors and four ventilation openings with opening and closing systems on each side of two axis railway wagons were noticed. Four axis wagons have double two folds, or single one fold doors, and four ventilation openings as well. Two double two folds and single one fold doors were constructed to biggest Italian wagons with area of 52m². Rings for animal binding were placed in 95% of two and four axis railway wagons. Barriers for animal separation and 200 litre water barrels were found on 52 German wagons which their total loading place surfaces of 33m². Some French railway wagons with surface of 42m² have two fold doors and eight ventilation openings with both sides opening and closing system, animal separating barriers, hay and fodder as well as drinking systems, some of wagons intended for suckling calves transportation have special equipment for animal watering and fodder prepare. Wagons were marked for transportation of 16 horses, 24 cows or fattened bulls, 80 calves and pigs, or 133 sheep. Floors were generally covered with metal, meanwhile rest parts were from the wood. Floors were well bedded, better than others. Ventilation openings on Yugoslav railway wagons can not opened regularly. Doors were partially or completely closed in summer, door openings were closed by wooden boards or wooden barriers preventing falling of animals and litter droppings (Photo 1).

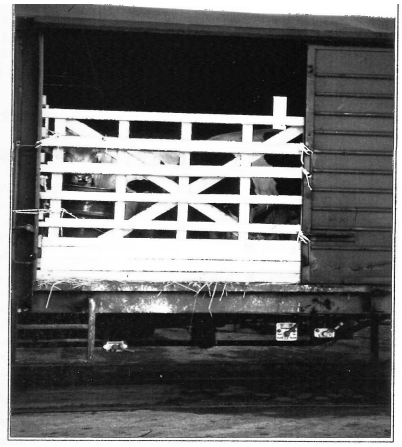

Photo 1. Wooden barrier on doors opening enable ventilation and prevent against animals falling and litter dissemination

Basic data which were taking into the statistical evaluation were conditions on animal transports. For evaluation we use statistical package SPSSX (Statistical package for social sciences). Consignments that were taken into the analysis vary from 4 to 98 animals, observation data for ostrich consignments were not statistically evaluated.

RESULTS

Table 1. Number of animals due to mode of transports and vehicle transport surfaces (m²) regarding animal species

Floor area (m2)	Cattle		Horses		Sum		
	road	railway	road	railaway	cattle	horses	Sum/Total
15	7.966	0	11.200	0	7.966	11.200	19.166
25	1.866	0	17.460	0	1.866	17.460	19.326
27	0	0	0	0	0	0	0
33	0	6.012	0	1.428	6.012	1.428	7.440
35	69.443	0	2.310	0	69.443	2.310	71.753
40	0	60.234	0	127.516	60.234	127.516	187.750
41	0	21.395	0	8	21.395	8	21.403
45	538	0	417	0	538	417	955
52	0	532	0	0	532	0	532
55	91.738	0	1.135	0	91.738	1.135	92.873
Sum	171.551	88.173	32.522	128.952	259.724	161.474	421.198

Legend:

Lorries (m^2) 15, 25, 35, 45, 55
R. car. (m^2) 27, 33, 40, 41, 52

Animal number due to categories

In the time of experiment 409.356 animals, what mean 96,4%, were going to slaughter (248.008 of cattle and 161.348 horses). Animals transported for further breeding have just 1% in share (4198 animals) what means 3888 cattle and 310 horses, calves for breeding have 2,6% in share (11.053 animals) of total number of transported animals. We follow 972 ostrich in transports as well.

Mode of transport and live weight

Most of horses have body weights from 250 to 400 kg, cattle weights were higher from 600 to 800 kg. Transport analyse proportions were almost equal when we analyse transports by the road where very heavy cattle were not transported. If we compare road and railway transport no significant differences can be noticed on horse transports.

Animal weight on m² of transport vehicle

Cattle consignments by road rich the highest point of stocking density when they weigh 250 to 400 kg. On railway they rich the highest point when they weigh 150–200 kg, and the lowest one when rich> 400 kg to m^2 of surface of transport lorry platforms. Most horses in road consignments rich the highest point when they weigh 250–300 kg, the lowest one when they were < 150 kg, meanwhile horses has to be 350–400 kg to rich the highest, and 200–250 kg to rich the lowest point of stocking density on the railway wagons.

Animal over plus in consignments regarding rule normatives (10)

When cattle were transported by road, relatively small numbers of consignments were according to rule normatives (10). Animal over plus was mainly up to 1 to 6 animals in vehicle. On the other hand most of cattle consignments by rail were within bounds of rule. If the rules were over crossed than it happens equally in all categories.

Table 2. Animal over plus in consignments regarding rule normatives (10), modes of transports and animal species (1980–1995)

Surplus	Cattle		Horses		Sum		Sum/Total
	road	railway	road	railway	cattle	horses	
0	621	2.584	77	1.162	3.205	1.239	4.444
1 - 3	1.801	233	519	1.617	2.034	2.136	4.170
4 - 6	1.740	248	737	2.128	1.988	2.865	4.853
7 - 9	1.236	120	443	507	1.356	950	2.306
more of 10	569	204	284	104	773	388	1.161
Sum	5.967	3.389	2.060	5.518	9.356	7.578	16.934

Lack of platform space in transport vehicles due to rules (10)

Cattle – 0,3 to 0,4 m^2 of space was insufficient for each animal at the most. Among cattle transports the lack of space was approximately equally distributed. On the road and in lorries the lack of space vary from 0,3 m² to 0,4 m² while on the railway wagons negative space lacks for cattle were relatively small.

Horses – Among horse transports lack of space was distributed approximately equally under all categories, highest point was in lacking surface of 0,6 m² to 0,7 m² for individual animal. Oh railway most of lacks of space vary up to 0,5 m² to each animal. Bigger lacks of space for more than 0,5 m² happened just in few cases no matter for cattle or horses.

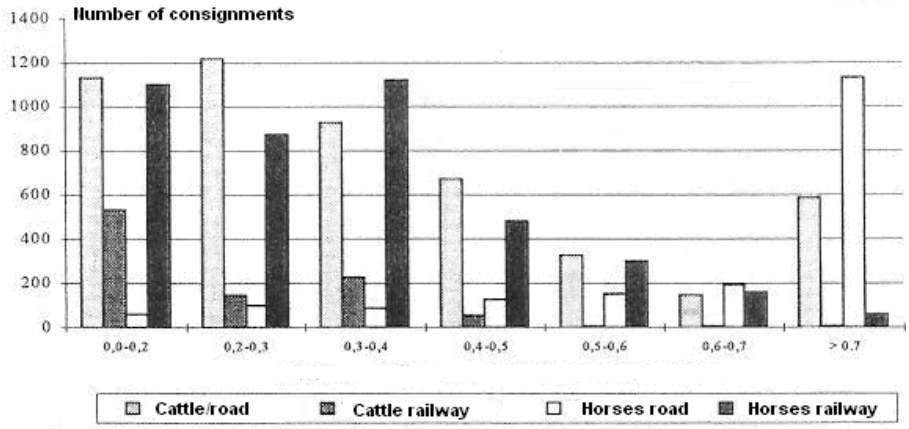

Figure 1. Number of consignments where animals has lack of space (m2) regarding rule normatives (10)

Cattle-road – most of consignments were 110 to 120% overcrowded. A bit less than 150% overcrowding can be noticed in 977 consignments.

Cattle-railway – most of consignments were transported in the normal range < 100%, just in 39 consignments where 1898 animals were transported it can be noticed < 150% overcrowding.

Horses-road – most of consignments were < 150% overcrowded where 19.086 animals were transported in 39 consignments. Just in 93 cases overcrowding did not exceed < 100%.

Horses-railway – most of consignments were 110–120% overcrowded, > 150% in 67 consignments – 2279 horses.

Ostrich

Ostrich were EU origin and designated for export in third countries.

Adult ostrich – were transported in vehicles intended for cattle or horse transportation. The average of animal altitudes was cca. 200 to 220 cm. Up to 12 consignments just in four of them, animals were regularly fed and watered. Three of ostrich consignments of live weight of 100 kg were transported in two floors, which altitudes were not more than 1,6 m, so animals were not in condition to stand in natural position. 10% of animals were obviously exhausted and in lying position due to the force. Animals in standing position beat the heads into the roof. Scrapes and bleeding were noticed on heads and animal bodies. Among 12 consignments just 1 was transported due to the prescribe rules in R Slovenia and EU.

Young ostrich – among 8 consignments (810 young ostrich) just 3 (113 animals) were in proportion with rules. We can notice just old wounds and lesions probably from the time of breed.

CONCLUSIONS

We can surely evaluate received results owing to data which were collected several years on large number of animals and several consignments. In the analyse of 16.954 transport vehicles, 8027 lorries and 8907 railway wagons there were 426.079 animals which were transported. From those analysed 248.008 cattle and 161.348 horses for slaughtering, 3088 cattle, 11.053 calves and 310 horses for further breeding, so as 662 adult ostrich in 12 lorries and 810 young ostrich in 8 lorries for further breeding, transported in periods from 1980 to 1995 and 2003 to 2004. Those transports were strongly connected by animal distress, pain, fear and other problems connected to bad transport conditions. We assume several mediate harms due to non suitable transports as well.
We should generally conclude that rules (10) which were comparable with EU directives (11) were not taken into the consideration in vehicles arrangement, stocking density and modes of transports notwithstanding rules and laws (12, 13) where long international transport conditions were clearly prescribed.

Our opinion is that for improvement of transport circumstances can be rational:

- to limit animal transports what mean slaughtering close to breeding place as possible
- to transport meat and meat products instead of live animals
- to stimulate animal suitable vehicle construction
- to educate organisers, drivers and long transports companions permanently
- to proceed research work on animal transports as well as contribution of improvement suggestions

– to establishing permanent evidence about direct and indirect harms on animals, generated in internal and international transports to strict considerate valid legislation on long international animal transport

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BLOOD CRITICAL VALUES´ PROFILE OF AMERICAN BARROWS *VS.* BOARS ARRIVING TO MÉXICO AFTER 27 HOURS OF TRANSPORTATION

Becerril-Herrera, M.[1], Jaramillo, P.[2], Rodríguez, R.[2], Alonso-Spilsbury, M.[2], Lemus, C.[3], Guerrero-Legarreta, I.[4], Olmos, S.A.[2], Ramírez-Necoechea, R.[2] and Mota-Rojas, D.[2]

[1] Student from the Doctorate in Biological Sciences, Universidad Autónoma Metropolitana. Iztapalapa/Xochimilco. [2] Laboratorio de Etología, Producción Porcina y Fauna Silvestre. Dpto. de Producción Agrícola y Animal, Universidad Autónoma Metropolitana-Xochimilco. [3] Facultad de Medicina Veterinaria y Zootecnia, Universidad Autónoma de Nayarit. [4] Laboratorio Bioquímica de Macromoléculas. Dpto. de Biotecnología. Universidad Autónoma Metropolitana-Iztapalapa.

ABSTRACT

Introduction

Transport, fasting, slaughter and thirst are stress factors contributing to live weight loss and poor carcass performance in pigs. Physiological responses of pigs as a consequence of transportation result in physiological stress and/or physical fatigue, and can even lead to death.

Keywords: pigs, transportation, haematocrit, carcass pH, boars, barrows, blood gases

OBJECTIVE

The aim of the study was to characterize and compare the blood critical values of barrows and pig boars transported from USA to Mexico in 27 hours period.

METHODS

A total of 90 Duroc x Pietrain pigs (40 barrows and 50 boars) coming from USA to Mexico with a transportation time of 27 hours were monitored from arrival to the slaughterhouse to sale as cold carcass. All pigs were blood sampled from the jugular vein within 15 sec of restraining on arrival. Blood was mixed with lithium heparin in order to impede alterations on blood gases. Acid-base imbalance, metabolic profile, and dehydration measurements were measured monitoring the following indicators: pH, bicarbonate, glucose, lactate, minerals, blood gases (pO_2 and pCO_2) and haematocrit, carried out with a critical blood value measuring device of third generation (GEM Premier 3000 from IL Diagnostics, USA-Italy). Results were analyzed through a Kruskal-Wallis test.

RESULTS

There were significant differences between male class (P<0.05) for: ear temperature (ºC), pH, pCO_2, Ca^{++}, glucose and haematocrit. On arrival at the abattoir, castrated male pigs had greater

(P<0.01) plasmatic Ca^{++} (mmol/L), glucose (mg/dL), pCO_2 (mm/Hg), and a higher temperature (°C) compared with the boars (Ca. barrows 1.29±0.01, boars 11.22±0.008; glucose: barrows 121.60±5.10, boars 66.12±2.76; pCO_2 barrows 41.50±1.25, boars 34.56±0.75, temperature: barrows 38.42±0.16, boars 37.67±0.19). There was a significant drop on pH values (P< 0.0001) for the barrows.

CONCLUSIONS

Results indicate that boars are more tolerant to transport stress than barrows. With regard to the haematocrit, boars showed higher values than barrows (46.28±0.83% vs. 39.95±1.06, respectively). It is worth mentioning that lactate levels (mg/dL) were within the normal range for barrows and boars (27.58±3.42 and 30.20±2.77, respectively). These findings may be due to the long transportation time where animals passed from the alarm phase with lacto-acidaemia to the adaptation phase where the balance for the respiratory chain for the ATP production by anaerobic route is restored.

LOGISTICS AT TRANSPORT TO SLAUGHTER. FOOD AND ENVIRONMENT – OPTIMISED ANIMAL TRANSPORT

Wiberg, S.[1], Algers, A.[2], Algers, B.[1], Franzén, U.[3], Lindencrona, M.[3], Moen, O.[3], Ohnell, S.[3] and Waidringer, J.[3]

[1] Swedish University of Agricultural Sciences, Department of Animal Environment and Health;
[2] Swedish University of Agricultural Sciences, Department of Food Science;
[3]WSP Analys och Strategi

SUMMARY

All kinds of transportation are a potential stressor to animals. Animals exposed to stress have reduced welfare. Stress can also lead to inferior meat quality and condemned carcases, which incurs both economical and environmental losses. The transports themselves also have a negative impact on the environment. A small scale and a medium scale abattoir were compared. The transport optimization in this pilot study shows good possibilities to improve efficiency by collecting the same amount of animals in a shorter period of time with reduced distance driven.

Keywords: animal welfare; animal transport; logistics; emissions; abattoir

INTRODUCTION

There is an increasing consciousness of animal welfare in food production and societal demands on the transport system are high regarding animal welfare and environmental impact. Research show that transports can be detrimental to animals leading to reduced welfare. The transports can cause stress and injuries on the animals which also can affect the meat quality (Atkinson, 2000). The profitability in the Swedish meat industry is low and at the same time, the slaughter industry is moving in a direction toward fewer and larger abattoirs with increasing areas of service.

The work presented here was conducted as a pilot study. The aim was to investigate the possibilities to optimise transport to slaughter in small and medium scale abattoirs, to improve transport conditions for the animals with a simultaneous decrease in environmental load and transport costs.

MATERIAL AND METHODS

A small scale (SA) and a medium scale abattoir (MA) were compared. Data on animal welfare and transport routes and routines were collected in June-August 2006 through questionnaires and visits to the abattoirs. At the abattoirs distance, times, number of animals, etc. was recorded. This data was used in both an animal welfare analysis and in a transport simulation. The latter also served as basis for environmental impact calculations where a comparison between the performed and the optimised transports was made.

The SA slaughtered once a week, and data was collected for five days. A large part of the animals slaughtered at the SA were reared at the farm where the SA was situated. The transports were conducted with 10 vehicles and 9 transporters. Most transports were performed by the owners of the animals. Two vehicles belonged to the farm and were used for transportation of their animals. The total number of collections of animals studied was 23 divided in 22 rounds.

Data from the MA was collected for two weeks (10 days of slaughter). Records from 178 collections divided in 85 rounds were obtained. Of these, 101 were collections of cattle, 76 of pig and 1 of sheep. Since there is only one recorded collection of sheep no calculations were made on transport of sheep. The transports were conducted with 17 vehicles. Of the vehicles, two belonged to the abattoir, four to farmers and the rest to private hauliers.

In the questionnaire, farmers, transporters and representatives from the abattoirs were asked about the attitudes towards a number of different changes that could make the animal transport system more optimal.

RESULTS

Vehicle 1–4 each performed between 17 and 26% and together 81% of the collections. As seen in figure 1, these four vehicles transported almost all cattle to MA. Figure 1 also shows how the transports of pig were distributed among the vehicles, and that vehicle 5 and 7, despite few collections, transported a large part of the pigs, and that vehicle 3, despite many collections, transported relatively few animals.

The proportion of animals, lairaged overnight was high, especially for pigs but also for sheep at SA (fig 2).

To SA all transports but one collected animals at only one farm, as most transports were conducted by the owners themselves. Of the 85 rounds to MA, 48 (56%) consisted of one collection.

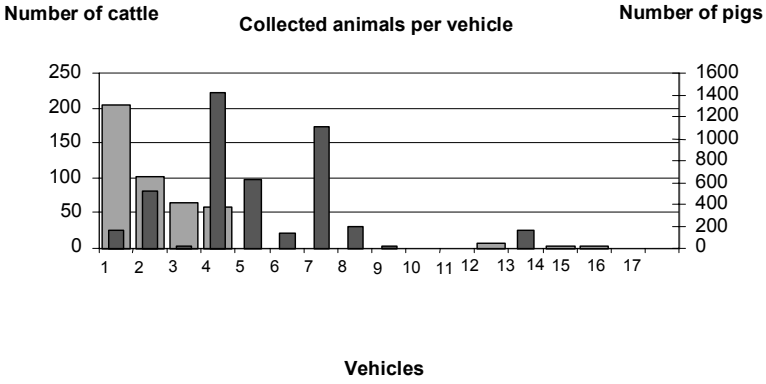

Figure 1. Animals transported to MA (Pigs=dark, cattle=light)

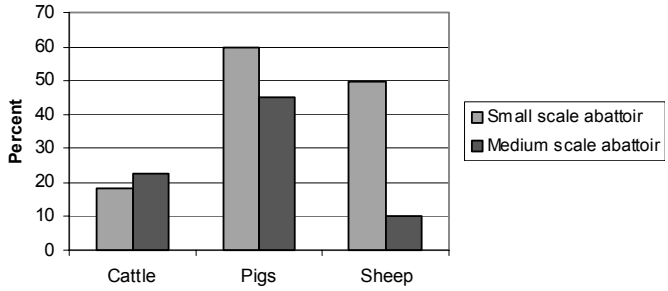

Figure 2. Animals kept in lairage overnight

The duration of handling at transport to slaughter varied considerably between abattoirs and species (table 1).

Table 1. Handling duration at transport to slaughter

	Cattle		Pigs	
	SA	MA	SA	MA
Time 1, (h:min)	00:00	00:04	00:00	00:05
Time 2, (h:min)	00:04	00:03	00:05	00:01
Time 3, (h:min)	00:01	00:05	00:02	00:05
Time 4, (h:min)	00.09	02:36	00:26	02:11

Time 1: Time from arrival of vehicle to start loading of animals; Time 2: Time for start to finished loading; Time 3: Time from finished loading to start driving; Time 4: Total travelling time

Mixing of animals was recorded on the transport and upon arrival to the abattoir. Transporters at SA answered that 50% (n=6) of the cattle, 0% (n=2) of the pig and 33% (n=9) of the sheep were mixed in the transport vehicle. The corresponding numbers for MA were 19% (n=75) and 78% (n=46) for cattle and pig, respectively. Mixing in lairage was not reported from SA, but recorded in 82% (n=22) and 100% (n=25) for cattle and pig at MA, respectively.

Group size at collection of cattle to SA were in 7 of 9 occasions 2–3 animals and in two cases 5–6 animals. Pigs were collected in groups of 3–5 animals in 4 cases and sheep <10 in 4 cases, 11–20 sheep in 3 and >20 sheep in one case. To MA, one single cattle was collected in 31% of collections. In 34% of the collections, the groups were of more than three animals. Pigs were collected in groups of 1–10 animals in 36% of the cases.

Optimised transports

The traditional way of transport planning is done with pen and paper. Since 2–3 years, there is a national digital database of all roads in Sweden, and their driving restrictions. This, in combination with GPS navigators and route optimizing computer programs, are tools that can revolutionize transport logistics.

For animal transports, there are several conditional elements that need to be considered to accomplish a good transport; e.g. legislation, overnight lairage, transport conditions, access to animal and access to vehicles and transporters. But there is also an advantage compared to many other parts of industry – a large “time window” for the collection of goods. A pig ready for

slaughter has about a five day interval where slaughter can be done before it gets too heavy. For healthy cattle, the interval is about three weeks, which is about the time the farmer accepts to wait. This means there is five days and 3 weeks, respectively, to plan the transport to slaughter. In the slaughter industry, the planning can be done during regular working hours with good planning in advance.

The optimizations in this study were done using the data from MA. At present, transports to MA are performed using 17 vehicles with different starting points; some start at the abattoir, others where the transporters live.

The first scenario, "Present" was done to recreate the performed transports as they were done in reality, deleting the five transport vehicles which only marginally contributed to the transports. Some information from the forms that were filled out at the visits to the abattoirs was used, and some basic conditions were set up, for example: Vehicles started at their present starting points and returned to the same place at the end of the day, the number of collections of animals was 178 and the time to unload and wash the vehicles was estimated to 50 min. In the second scenario, "Optimized present", a computer program was used to optimize the transports. The program could choose what day and what vehicle to use for each round. The basic conditions were, for example: Only the largest vehicles were used, the vehicles started at their present starting points and returned to the same place at the end of the day, maximum transport time of eight hours and no animals kept in lairage over night. A third optimization was done again, "New optimization". Here, the basic conditions were still maximum transport time of eight hours and no animals kept in lairage over night, but also that only five of the largest vehicles were used and that the vehicles all had the same starting and return point (the abattoir). See table 2.

Table 2. Different scenarios after optimisation

	Present	**Optimised present**	**New optimisation**	**% reduction present – new optimisation**
Vehicles, n	12	7	5	– 58%
Distance, km	14153	12167	9894	– 30%
Time, min	26720	22988	20653	– 23%
Rounds, n	85	51	58	– 32%
Time/round, min	326	390	356	+ 9%
Distance/round, km	167	239	171	+ 2%

Environmental impact

The available data on transports to the SA was too limited to do reliable calculations on emissions. For the MA, calculations on emissions have been made for the scenarios "Present" and "Optimized present".

Calculations on emissions are based on the transporters data on loading capacity, fuel consumption and driving distances. Data on emissions from vehicles of different Euro classes are collected from "nätverket för transporter och miljö" (2007).

The optimization shows large potential to reduce emissions, see table 3. The CO_2 -emissions are reduced in relation to driving distance. Thus what is effective from a commercial viewpoint also reduces the environmental impact. To decrease emissions further (NOx, HC and particles), replacement of old vehicles was shown to have the largest effect. Subsidies or legal demands are ways to speed up such replacements.

Table 3. Emissions as % reduction of scenario "Present"

	Optimised route	**Euro 2**	**Euro 3**
Distance	11%	11%	11%
HC	20%	34%	42%
NO_x	16%	4%	27%
PM	20%	40%	42%
CO_2	12%	12%	12%

Attitudes

The studies on attitudes show that it is a general belief that picking up animals earlier in the day to avoid overnight lairage is positive for animal welfare and that the use of pre loading facilities is viewed as being positive for animal welfare and labour situation. Producers of slaughter animals believe that animal welfare can be improved by the use of mobile slaughter facilities. There is a positive attitude to transporters given their own geographical region irrespective of the receiving abattoir and a negative attitude to several transporters working in the same area to increase flexibility.

DISCUSSION AND CONCLUSION

A large part of the collections of animals were done in small groups of animals. To make use of vehicles with large loading capacity and collecting small groups of animals mean more stops per round. Many stops are negative for animal welfare (Gebresenbet and Eriksson, 1998). Small groups also increase transport time and the risk of mixing animals.

It was common to mix animals in the transports and in lairage. To transport small groups of animals under conditions with good animal welfare and at the same time use the vehicle capacity, the equipment for fencing the animals in the transport needs to be flexible.

Loading is, for good animal welfare, the most crucial moment of the day of slaughter (Fraser and Broom, 1990). Loading time differs between the two abattoirs, and is for example longer for pigs for the SA. A longer loading time is positive for animal welfare, as the animals can walk at their own pace (Hemsworth, 1993). Studies have shown that animals that are mixed in the transport vehicle are exposed to stress (Bradshaw et al, 1996). A short time from arriving at the farm until start of loading is therefore beneficial for animals in transports which previously collected animals. The differences in times 1–4 (table 1) between SA and MA are due to the number of animals and the way the animals are transported. At SA, they are in most cases driven across the farm yard by their owners, and at MA, professional transporters with a tight schedule and large vehicles transport a larger number of animals.

Time from arrival at the abattoir until slaughter varied greatly because some animals were slaughtered immediately after arrival, while others were kept over night. A large part of the animals at both abattoirs are kept in lairage over night. After a well performed transport there are no advantages in keeping animals in lairage and a long time in lairage increases the risk of spreading contagious diseases (Warriss, 2003). Animals exposed to stress can benefit from around two hours in lairage, under good circumstances (Santos et al., 1997). Time in lariage is apart from that considered a factor of stress and should be avoided (Geverink et al, 1998; Santos et al., 1997).

In the optimized scenarios, mean travel distance per animal increase compared to "present". Further research is needed to analyse the effect of an increased number of stops and longer

journeys compared to long and overnight lairage on animal welfare. Further research is also needed to study the effect of flexible interiors in the transport vehicles to reduce mixing and thus the stress load on the animals.

Emissions are reduced as the distance decreases. To further reduce emissions it is the exchange of vehicles that induces the largest effects.

The conclusion from this pilot study is that transport optimisation can result in simultaneously increased animal welfare, reduced costs and reduced environmental impact. Farmer attitudes show openness to such changes.

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THE DEVELOPMENT OF A STUN QUALITY AUDIT FOR CATTLE AND PIGS AT SLAUGHTER

Atkinson, S. and Algers, B.

Swedish University of Agricultural Sciences, Department of Animal Environment and Health

SUMMARY

Swedish regulations for stunning of cattle and pigs stipulate that the time interval between stun to stick should be within 60 seconds. There are difficulties for many abattoirs to achieve this due to the technical design of the shackle line. An auditing system was developed by the Swedish University of Agricultural Sciences to perform externally conducted assessments investigating stun quality in relationship to stun to stick interval times.

In total 2700 cattle in 5 abattoirs and 8941 pigs in 7 abattoirs were studied. There were no cattle abattoirs that had a 100% effective stun rate, but 92% of all cattle were considered deeply stunned. Reasons for failure to stun properly in cattle abattoirs were identified mainly to be related to the stun weapons used.

Five of 7 pig abattoirs achieved a 100% deep stun effect. Two abattoirs were re-audited due to some pigs displaying corneal reflex. Reasons for poor stun effect were contributed to too low CO_2 exposure which was solved by slightly increasing the CO_2 exposure time and CO_2 gas concentrations.

At all pig and cattle abattoirs stun to stick intervals exceeded 60 seconds. Only 30% of all pigs and 5% cattle audited were stuck within 60 seconds. When animals are deeply stunned, the stun to stick interval is less critical. However, auditing results detected problems with achieving short stun to stick intervals and a 100% stun effect. This highlights the importance of good stunning practice as well as the use of a stun auditing procedure.

Keywords: animal welfare; stun quality; cattle; pigs; slaughter

INTRODUCTION

To ensure good animal welfare during post stun stunning, unconsciousness should be induced of a sufficient duration to include not only the stun stun-stick interval but also the time taken for the animal to become insensible due to de-bleeding, which must be started as soon as possible (93/119/EC). A good stun will however make the time interval less critical. The slaughter operations in abattoirs vary considerable due to different technical designs, different stun systems, and different killing rates.

In Sweden, the slaughter industry in response to societal demands started to upgrade standards for handling and stunning, including installing automatic driving of pigs in groups and automatic driving of cattle into the stun pen. Swedish regulations for stunning of cattle and pig species stipulate that the time interval between stun to stick should be within 60 seconds. There are difficulties for many abattoirs to achieve this due to the technical design of the shackle line which cannot transfer carcasses to the sticking area in less than 60 seconds. This is especially the case for newer designs of CO_2 group stunning of pigs, where up to 10 pigs can be stunned at a time. A

demand for externally conducted assessments arose by the abattoir industry in Sweden to investigate stun quality in relationship to stun to stick interval times. As a part of this, evaluations of stun quality were developed and stun quality audit programs were implemented. This paper describes the process of ensuring welfare standards at slaughter in Sweden through the development of a stun quality audit protocol and its use in 7 pig and 5 cattle abattoirs. It also highlights how critical stun to stick intervals may be for cattle when using captive bolt stun and for pigs when using CO_2 gas stunning.

METHOD AND MATERIALS

In total 2700 cattle in 5 abattoirs and 8941 pigs in 7 abattoirs were studied. For both cattle and pigs information was collected on the design of the stun to stick areas, the stun system used, the stun to stick intervals and the stun quality. The stun quality was assessed by recording the symptoms displayed by each animal after stunning until the point of sticking. Stun to stick intervals were recorded using a stop watch and analysed for minimum, maximum and average time intervals. For cattle the start of the stun was timed as soon as the shot was heard from the stun gun. For pigs it was started at the point where the stun box opened to release the CO_2 stunned pigs. The stun to stick interval was timed for every pig in the group. Sticking for both species was considered to be the point at which the knife was pushed into the throat of the animal for de-bleeding. The following information for cattle was recorded on a set Pro Forma;

- the number of times each animal was shot
- type of animal being shot i.e. sex, age, breed
- physical reactions and reflex symptoms on the animals that could indicate a bad stun
- any incidents or stops in the system between stun to stick
- stun to stick intervals

A similar Pro Forma was completed for pigs, but included group sizes and stun to stick interval times for every pig in the stun group. Details of the gas procedure such as number of boxes in the system, box rotation times, gas chamber depth and CO_2 exposure times were also measured.

Animals that were suspected of not being stunned properly were closely examined and symptoms noted. The eyeballs and corneal area were touched to watch for any eyelid blink. If there was a reaction this was considered as a corneal reflex. Stun quality details were grouped into 3 categories (Table 1) which identified the stun quality as being either good, poor or undefined i.e. a stun that does not clearly fit into good or bad stun categories.

Table 1. Symptoms that indicate stun quality level

Stun Quality	Symptoms
Deep or good stun	– Dilated pupils – No eye ball rotation – No corneal reflex – Minimal kicking and reaction to sticking procedures
Poor stun	– Corneal reflex – Spontaneous blinking – Breathing/respirations – Full or partial eye ball rotation up to sticking
Undefined stun quality but separating stun from deep or poor stun quality symptoms	– Gasping, groaning – Excessive kicking or struggling at sticking in combination with above symptoms in this category

For cattle, any symptoms other than a deep stun effect were rated in seriousness from 1 to 4 (Table 2), with ratings 1 or 2 indicating low stun quality (rate 1 being the lowest i.e. corneal reflex present, and rate 2 reflex indicating spontaneous blinking and or fixed eyeball rotation). The reason this was developed was because cattle tended to show more and stronger symptoms than pigs post stunning, due to the physical damages caused to the brain from being physically hit which evokes many uncontrolled neural reflexes compared to the anaesthetic effects after CO_2 gas stunning. Symptoms such as eye ball rotation could also disappear and deep stun symptoms appear after a few seconds post stunning. Therefore it was decided that the variation of symptoms should be rated, to help with stun quality assessments.

Table 2. Symptoms of a poor stun effect used for cattle

1	– Corneal reflex
2	– Spontaneous blinking – Full or partial eyeball rotation up to sticking
3	– Full or partial eye ball rotation followed by pupil dilation before sticking
4	– Gasping, groaning – Excessive struggling or kicking at sticking

RESULTS

The development and implementation of the auditing program showed that there was a large variation between both pig and cattle abattoirs in respect to design of the stun system, but also in stun to stick intervals and stun quality (Table 3).

Table 3. Average stun to stick intervals and the stun quality results for all audits

Cattle	% animals with stun to stick interval less than 60 seconds	% Animals deeply stunned	% Animals poorly stunned	% Animals with undefined stun
Audit 1	0	88	10	2
Audit 1b*	2	94	2	4
Audit 2	1	93	5	2
Audit 3	15	96	2	2
Audit 4	1	96	2	2
Audit 5	68	98	1	1
Pigs				
Audit 1	65	100	0	0
Audit 2	59	98	2	0
Audit 2b*	75	100	0	0
Audit 3	94	97	3	0
Audit 3b*	82	100	0	0
Audit 4	43	100	0	0
Audit 5	64	100	0	0
Audit 6	1	100	0	0
Audit 7	21	99.8	0.2	0

*Abattoirs that were re-audited

One cattle abattoir used a pneumatic powered stun gun, 2 abattoirs used guns that fired free bullets for larger bulls, and the rest used only captive bolt guns for all cattle classes. No cattle abattoir had a 100% effective stun rate, but the abattoir with the best stun quality (98% well stunned), used the pneumatic stun weapon. Of all cattle audited, 92% were considered deeply stunned.

Three pig abattoirs had a 1 box stun system, each with different capacities for stun group sizes of 3,5, and 7 pigs. Two abattoirs had 4 boxes, 1 holding 3 pigs, and the other 5 pigs per stun group. The largest abattoirs had 6 and 7 boxes in the stun system, and could stun 5 to 10 pigs respectively. CO_2 concentrations ranged from 91 to 93% during stunning, and the exposure times ranged from 1 minute 54 seconds to 8 minutes. Five of 7 pig abattoirs achieved a 100% deep stun effect. Two abattoirs were re-audited due to a number of pigs displaying corneal reflex (3% in one abattoir, 2% in the other), but after re-auditing 100% of pigs were deeply stunned. Of all pigs audited, 99.4% were considered deeply stunned. After re-auditing, all seven pig abattoirs had such good stun quality that the fact that the stun to stick intervals were longer than 60 seconds were considered to be less critical from an animal welfare perspective.

DISCUSSION AND CONCLUSION

The results of these audits showed that few abattoirs can consistently achieve stun to stick intervals within 60 seconds. However, good stun quality was achieved despite this, especially in pig abattoirs where stun to stick intervals over 60 seconds could be considered less critical. There were more problems with stun quality in cattle abattoirs. Reasons for failure to stun properly were contributed to shooting outside the area required to cause appropriate brain damage, use of unclean or unserviced guns (with worn out parts) and use of damp ammunition. In all audits bulls were more difficult to stun properly than any other cattle even if shot correctly (up to 11% of bulls were poorly stunned in 1 abattoir).This was attributed to bulls having thicker skulls and the use of too weak weapons. In 2 abattoirs more powerful weapons were available for use on larger bulls, (9.6 calibre free firing bullet, compared to a 0.22 calibre retractable bolt). This gun type however, is more dangerous to use for staff and is therefore used as minimally as possible. To ensure proper stun quality for bulls, it would be better welfare to use it more often, or invest in another type of weapon. Weapons used in abattoirs in Sweden for large cattle should be reconsidered as well as the training of the people performing the stunning. If the stun effect is good and lasts for several minutes, then the stun-to stick interval becomes less critical; though, immediate bleeding ensures better welfare.

Reasons for poor stun effect in pigs were contributed to too low CO_2 exposure times for each stun group which was solved by increasing the CO_2 exposure time by just 16 seconds, and slightly increasing CO_2 gas concentrations from 91% to 93% within the stun box chamber. There was also an occasion where there were too many pigs for the size of the stun box, and upon observation of pig behaviour during the stunning, the pigs could fall on top of each other, and in some cases their head could be higher than the ground level, which could be a reason for some pigs having a reduced stun effect. This especially seemed to occur during the stunning of sows which were much larger than the prime slaughter pigs.

In many cases pigs that displayed corneal reflex, either kicked or gasped as well. Therefore it was recommended to the abattoirs that any pigs showing gasping or kicking at any time during the stun to stick interval, and even after sticking, should be checked and re-stunned if required.

This study has shown that some cattle can rotate the eyeball immediately after stunning or after some 10 or 20 seconds, after which it centres, the pupil dilates and the animal shows symptoms of being properly stunned. The anatomical basis of the conscious state is also not well understood, and according to Finnie et al (1997) it depends on feedback loops of neural activity between the brainstem reticular activating system and the cerebral cortex (Finnie et al 2002). Gregory and Shaw (2000) discusses current scientific knowledge about whether penetrating captive bolt stunners applied to the frontal areas of the head reliably cause loss of consciousness in cattle, and how to assess the risk of recovery of consciousness. They mention that physical responses to some types of nociceptive stimuli can occur at both conscious and subconscious levels, and can add complications when attempting to establish whether an individual is insensible to pain. This study highlighted the fact that animals can display many different symptoms after stunning. Thus, in order to assess stun quality is a difficult task. Therefore, by implementing a protocol for investigating stun quality in cattle and pigs at slaughter, more consistency was achieved.

In conclusion, the results of the audit did identify certain problems in stun quality in some abattoirs. The abattoirs were then able to use this information and make changes which could be shown to improve animal welfare at slaughter.

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STUN QUALITY IN RELATION TO CATTLE SIZE, GUN TYPE AND BRAIN HAEMORRHAGES

Algers, B. and Atkinson, S.

Swedish University of Agricultural Sciences, Department of Animal Environment and Health

SUMMARY

Data collection was made on 594 cattle to assess stun quality. Brain damage was assessed on 14 of these cattle. The brain bleedings were limited to cattle showing corneal reflex. The largest bleedings (located at the stem and base of the brain); were seen in cattle shot with a pneumatic stun gun. The results suggest that causes of poor stunning included inaccurate shooting on the skull and the use of a too weak weapon for bulls. Heavy blood haemorrhage or brain matter disintegration in the frontal regions of the brain, do not necessarily indicate unconsciousness or insensitivity to pain.

Keywords: animal welfare; stun quality; cattle; slaughter

INTRODUCTION

The captive bolt is the principle method of preslaughter stunning of cattle. It is intended to produce instantaneous insensibility until death occurs from exsanguination. However, Finnie et al (2002) studied brain damage in sheep after captive bolt stunning and found that the severity of structural brain damage varied considerably. Daly (1991) studied stun effect on 2500 cattle and found bulls were more difficult to stun than other cattle classes. The captive bolt creates a large, deep, penetrating and well defined haemorrhagic track and, although the injury is penetrating rather than perforating, the bolt frequently traverses almost the full thickness of the brain. This study aimed to macroscopically examine the skull and brains of cattle that displayed symptoms of poor or uncertain stun quality, to determine if these symptoms correlated to inferior levels of brain damage compared to properly stunned cattle. The study also aimed to compare stun quality and associated brain damage in bulls shot with a captive bolt stun gun, and bulls shot with a pneumatic stunner.

MATERIAL AND METHODS

Data collection was made in 2 different abattoirs. In total 594 cattle were observed during stunning in abattoirs A and B. In abattoir A, a captive bolt stun gun was used, manufactured by Accles and Shelvoke LTD, type Cash Magnum 9000, 0.22 calibre. The gun had finger-activated trigger and fired a 121 mm long, and 11.91mm diameter retractable bolt. Three cartridge types were used to fire the bolt pistol according to the size of the animal and colour coded accordingly. Green cartridges (3G) were used for beasts under 300kgs live weight, red cartridges (4G) for animals over 300kg (medium sized animals such as cows, heifers and steers), and black cartridges

(4.5G) were used for very heavy bulls. The firing velocity and energy values according to the manufacturer's specifications for the Cash 0.22 calibre gun are as follows:

Black cartridges – 66.8 m/s and 517 joules. Red cartridges – 56.4 m/s and 361 joules. (Accles and Shelvoke LTD 2003; Detec, 2003). The captive bolt stun gun used in abattoir B was the same type as in Abattoir A. All bulls were shot with black cartridges. In Abattoir B, a pneumatically air operated and trigger fired stunner was also used, manufactured by Jarvis products corporation, USA, type USSS-1. The specifications for this gun were as follows: Operating pressure 11–12 bar. Air consumption per cycle 41 L. Penetrating bolt diameter 15.9 mm.

Stun assessments

Each animal was closely examined immediately after stunning and continuously up to sticking. Eyeballs in a fixed stare straight forward (no movement), dilated pupils and Minimal kicking and reaction to the sticking procedure outlines the clinical symptoms used to identify animals that were considered satisfactorily stunned. Cattle that showed symptoms separating them from the above protocol were recorded and rated from 1–4 (table 1). Symptoms rated 1or 2 were considered as indicating low stun quality (rate 1 been the lowest). Rate 3 and 4 symptoms were considered as important to note, but not as serious as rate 1 or 2 reflexes.

Table 1. Displayed symptoms grouped and rated from 1–4

Rating	Symptoms displayed
1	• Corneal reflex
2	• Spontaneous blinking • Full or partial eyeball rotation up to sticking
3	• Full or partial eye ball rotation followed by pupil dilation before sticking***
4	• Gasping, groaning • Excessive struggling or kicking at sticking

***Literature suggests that properly stunned animals should not show any eye ball rotation. However this study has shown that some cattle can rotate the eyeball immediately after stunning or after some 10 or 20 seconds, after which it centres, the pupil dilates and the animal shows symptoms of being properly stunned.

Macroscopic head examinations

Brain damage was assessed on a selection of cattle only. The selection was intended to make a comparison between cattle displaying symptoms of a deep stun, and those displayed a series of other symptoms which could indicate otherwise. Each selected animal was identified and the head taken from the slaughter line after decapitation and skinning. After examination all brain material was placed in the SRM (Serious Risk Materials) container for appropriate disposal.

Brain damage

The level of brain damage was examined, firstly while still in the skull. The bolt penetration wound into the brain cavity was recorded as hitting either low, midway, or high in the brain cavity. The brain was then removed from each half of the skull and further examined for amount and location of bleeding and damage. These damages were quantitatively assessed by estimating the percentage of the brain surface with blood haemorrhage.

RESULTS

The stunning of cattle in abattoir A and B when using the captive bolt guns resulted in poor stun quality in 1.9% of cows and 18% of the bulls studied. The use of a pneumatic stun in abattoir B resulted in a poor stun quality in 0.4% of the cows and 1.3% of the bulls. Table 4 shows a summary of the reflexes seen in both abattoirs for both gun types used. In total, 7% of the bulls shot with the captive bolt stun gun showed corneal reflex symptoms, while none of the pneumatic gun shot bulls had corneal reflexes.

Table 4. Summary and comparison of symptoms rated 1 or 2 in abattoir A and B with captive bolt weapon use in abattoir A, and captive bolt and pneumatic stun gun use in abattoir B

Abattoir	Gun type	Reflex rating 1		Reflex rating 2		Reflex ratings 1& 2	
		Cows	Bulls	Cows	Bulls	Cows	Bulls
A	Captive bolt	0/150 (0%)	3/100 (3%)	3/150 (2%)	12/100 (12%)	3/150 (2%)	15/100 (15%)
B	Captive bolt	0/10 (0%)	5/20 (25%)	0/10 (0%)	2/20 (2%)	0/10 (0%)	7/20 (35%)
B	Pneumatic gun	0/240 (0%)	0/74 (0%)	1/240 (0.4%)	1/74 (1.3%)	1/240 (0.4%)	1/74 (1.3%)

Large brain bleedings were considered to occur when there was bleeding down the centre of the brain and at the brain stem area. Minor brain bleedings were considered to occur when there was no or very little bleeding at the brain stem area, and down the central path of the brain. All cattle that showed corneal reflex symptoms (reflex rate1), had minor brain bleedings (figure 1), even though they were shot more than once. The brain bleedings were the least in cattle showing corneal reflex, and the largest brain bleedings at the stem and base of the brain were seen in cattle shot with the pneumatic stun gun (figure 2).

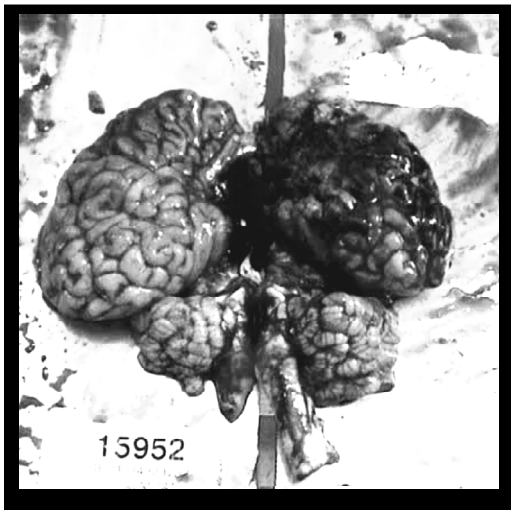

Figure 1. Captive bolt shot bull, shot 3 times.

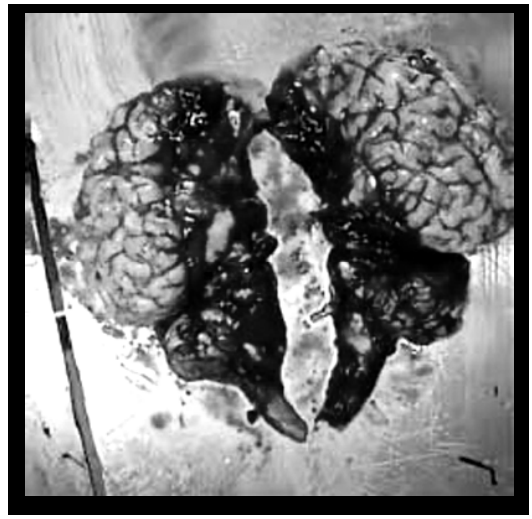

Figure 2. Pneumatic bolt shot bull, shot once.

The results suggest that the causes of poor stunning were inaccurate shooting on the skull and the use of a too weak weapon for bulls.

DISCUSSION AND CONCLUSIONS

In routine stunning at slaughter, a significant proportion of cattle, mainly large bulls, show signs of poor stun. The brain damages seen by the pneumatic gun tended to be larger, with more and heavier bleeding areas at the back of the brain. This suggests that the brain is shaken more vigorously with the use of the pneumatic gun at shooting, contributing to better stun quality. Bleedings on the brain as a result of a hit, tend to occur on the opposite part of the brain where the impact occurred ("contre-coup"-effect). To create a rapid and massive bleeding it is favourable to cause an arterial bleeding in the subdural or subarachnoidal areas around the brain stem and basal parts of the brain. As the arteries enter the brain at the base, that area is an important target to cause disruption and bleeding. Heavy blood haemorrhage or even brain matter disintegration in the frontal regions of the brain, do not necessarily indicate certain unconsciousness or insensitivity to pain. However, if there are bleedings around the brain stem and subarachnoid haemorrhaging at the base of the brain, there will be definite unconsciousness and a high probability of death. To reduce the risk of poor welfare at slaughter, the stunning of bulls with the use of guns must aim at resulting in such brain stem haemorrhages.

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CO_2 – STUNNING OF TURKEYS IN A V-SHAPED TUNNEL

Hänsch, F.[1], Nowak, B.[2] and Hartung, J.[1]

[1] Institute of Animal Hygiene, Animal Welfare and Behaviour of Farm Animals; [2] Institute of Food Quality and Food Safety, University of Veterinary Medicine Hannover, Germany

SUMMARY

Stunning with carbon dioxide gas (CO_2) is recently discussed as an alternative to electrical water bath stunning of turkeys. Investigations were carried out in order to assess some welfare aspects in turkeys when stunned with CO_2. The turkeys passed an increasing CO_2 atmosphere in a V-shaped tunnel on a commercial slaughter plant under practical conditions. The behaviour of the animals in the tunnel was observed and some clinical reflexes were checked before shackling. First results indicate significant convulsions immediately after entering the CO_2 atmosphere and deep unconsciousness without any clinical reflexes when leaving the tunnel.

Keywords: stunning, CO_2, turkeys, behaviour, animal welfare

INTRODUCTION

Turkey meat is a popular food not only in the EU but all over the world. About 1,890,000 t of turkey meat are produced in the EU, and even 2,464,000 t in the USA (1). The most common stunning procedure before bleeding is the electric water bath method even if there are some well known disadvantages (2).

In recent years it has been shown that exposition to gases like carbon dioxide (CO_2) can be an alternative eliminating welfare concerns related to some failure associated with the application of electrical current and the stress the animals suffer during shackling (3). The birds can remain sitting in their transport boxes and enter the gas stunning tunnel system without being stressed by human contact in the slaughterhouse. After the stunning procedure they do not realise uncrating and shackling because of being unconscious.

However, there are also disadvantages in relation to stunning with CO_2. Firstly, the birds go through a phase of excitation when entering the CO_2-atmosphere. This time period is not yet well defined but can last at least 10 s. Secondly, analgesia must continue beyond the point of neck cutting to make sure the animals do not gain consciousness again before having bled to death. Therefore it is required to keep the initial period as short as possible and reach unconsciousness as quick as possible. Proposals were made to divide the process of stunning into stages of different gas concentrations and different types of gases and leave the bird sufficiently long in such an atmosphere (4). One option is to work with an increasing CO_2 concentration in air during the stunning process from which a hypercapnic hypoxia results (5). Monitoring the state of consciousness of the animals during the stunning process is crucial. This can be done by testing e.g. the reflexes of the birds during the period of time from coming out of the tunnel until neck cutting.

This paper reports on some first results from stunning turkeys with CO_2 in a V-shaped tunnel observing their behaviour in the CO_2-atmosphere and checking their consciousness by testing

clinical reflexes (eye lid reflex, interphalangeal reflex) when leaving the tunnel and before shackling.

METHODS

The study was carried out in a medium sized typical poultry slaughterhouse in the north-east of Germany which stuns turkeys with CO_2 in a newly designed V-shaped stunning tunnel. Figure 1 shows a drawing of the system. The birds enter the tunnel sitting in their transport boxes (crates) by means of a mechanical conveyor belt directly from the transport lorry. Each box contains either five cocks or eight hens. One crate needs 180 sec to pass the tunnel. The process runs continuously from right to left employing two conveyor belts. The descending conveyor belt of the tunnel (YARA, Dülmen, Germany) is about 6 m long, the ascending belt 3 m.
CO_2 is injected in the tunnel at three points (Figure 1, points 1-3). At the end of the ascending part of the tunnel the anaesthetised birds fall on a conveyor-belt and are shackled upside down manually.

Gas monitoring: The concentrations of CO_2 and oxygen (O_2) were measured continuously for several hours on five different days in the tunnel atmosphere at different sites as indicated in Figure 1. CO_2 was measured at points B, C and D by an integrated device to monitor and correct the CO_2 concentration at the set points of 20%, 45% and 85%. Additionally we installed a second gas analysing system consisting of the gas analyzer unit "EL6010-Uras14" for CO_2 which measures per NDIR (Non-Dispersive Infrared Absorption) technique and the "EL6010-Magnos106" oxygen (O_2) analyzer (Figure 1, points A- E) which measures the specific paramagnetic behaviour of oxygen (ABB Advance Optima System, Zurich, Switzerland). Gas samples were sucked by continuously running pumps through Teflon tubes (4x1mm) from the sampling to the analysers. Five all-day measuring campaigns were completed so far.

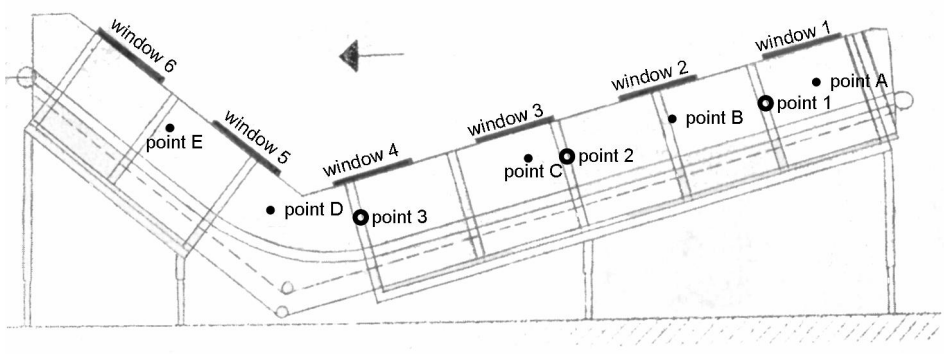

Figure 1. Outline of the V-shaped CO_2-tunnel for stunning turkeys. The gas measuring points from A to E, the gas inlet points 1 to 3 and the windows for observations from 1 to 6 are indicated

The animals: The study included 2830 (1717♀/ 1113♂) turkeys of the strain BUT Big 6. The behaviour was observed of 2600 turkeys (n_1) and 230 turkeys were tested for their reflexes (n_2). The average age of the males was 20 to 21 weeks and their weight about 20 kilos. The hens were slaughtered at an age of 16 weeks weighing seven to nine kg.

Behaviour: The animals` behaviour (n_1=2600) was studied by direct observations through the first four transparent glass windows (80 x 60 cm) in the ceiling of the tunnel (Figure 1). Each window section in the descending part was directly observed for about 100 minutes, divided into 50 min observation of hens ($n_{1.1}$=1600) and 50 min observation of cocks ($n_{1.2}$=1000). Following behavioural patterns were monitored: wing flapping, divided into light (only light single flaps), intense (flaps were more often and powerful) and excitative (excitations shown as long running flaps with a high frequency) flapping, deep breaths, head shaking, head´ s changeover into opisthotonus, lost posture of the head and preserved posture of the head respectively in high CO_2 concentrations (in window 4). It was not possible to watch every single animal in one box because the viewer´ s perspective was limited by the size of the windows, the moving crates and the sufficient but reduced light and by the fact that some birds were sitting one upon the other. Thus, the direct observation could not monitor every single animal but gives a good impression of the animals` behaviour in the areas with different gas concentrations.

Reflexes: Having passed the tunnel all indiscriminately chosen animals (n_2=230), 117 hens ($n_{2.1}$) and 113 cocks ($n_{2.2}$), were taken off the conveyor-belt short-time before the point of shackling. Painful stimuli were placed with surgical tweezers in the Telae interdigitales of both feet (Interphalangeal reflex) and the eyelid closure was controlled. The reactions to the painful stimuli were divided into negative (no reaction) and positive (reaction) results. Eyelid closure was discriminated from no closure (open eyes), full closure (closed eyes) and half opened eyes. After this procedure the birds were shackled to continue the normal slaughtering process.

RESULTS

Gas monitoring: Table 1 summarises the CO_2 and O_2 concentrations measured at the five sampling points A to E on five days by the ABB-gas analyser. Each figure represents the arithmetic mean of five measurements.

The lowest CO_2 and the highest O_2 concentrations are found at point A. At point C a concentration of 70 % CO_2 is observed. This is about 75 sec after the animals have entered the gas atmosphere in the tunnel. The highest CO_2 levels are reached at point D. Usually at this point the birds did not show any movement anymore. The O_2 level stayed at around 6 to 8 % from point C to D.

Table 1. Arithmetic mean (x) gas concentrations (CO_2, O_2) with standard deviation (s) at the five measuring points (A to E) in a stunning tunnel for turkeys in a commercial slaughter house

Gas	Point A	Point B	Point C	Point D	Point E
	x s	x s	x s	x s	x s
CO_2 %	27 ± 6,3	35 ± 5,5	70 ± 10,3	77 ± 11,2	72 ± 7,8
O_2 %	16 ± 1,4	14 ± 1,7	8 ± 2,0	6 ± 2,2	7 ± 2,0

Behaviour: Most of the turkeys are sitting in the crates while being carried to the tunnel's entrance, only a few are standing. Many birds show panting and breathing through the opened beaks. Because of the incline of the V-shaped tunnel the cages cant forward when entering the tunnel. Many turkeys stand up, patter and flap lightly with their wings as to keep balance. Figure 1 gives the percentages of observed head postures and reactions of turkeys during stunning with CO_2 at different positions in the gas tunnel. At the position of window 1 all birds were completely

conscious. More than 20 % started head shaking. 30 seconds later at window 2 about 7 % of the birds showed the head posture of opisthotonus. An increasing number of birds loose head posture completely but some preserve the posture of the head (5 % at window 4). In Figure 3 the frequency and intensity of wing flapping is illustrated at the different observation points. Light wing flapping can be seen at all windows with the highest incidence at window 1 (28 %) followed by window 4 (18 %) and lowest at window 2 (3 %). Observations of breathing showed that at window 1 about 2.2 % of the birds did a deep breath with an opened beak. This number increased in window 2 up to 11%, decreased in window 3 (1.5 %) and window 4 (1 %). No animal showed any reactions while coming out of the tunnel. All turkeys lay down with lost posture and predominant closed eyes. There were never any moves monitored during the process of shackling and before the point of neck cutting.

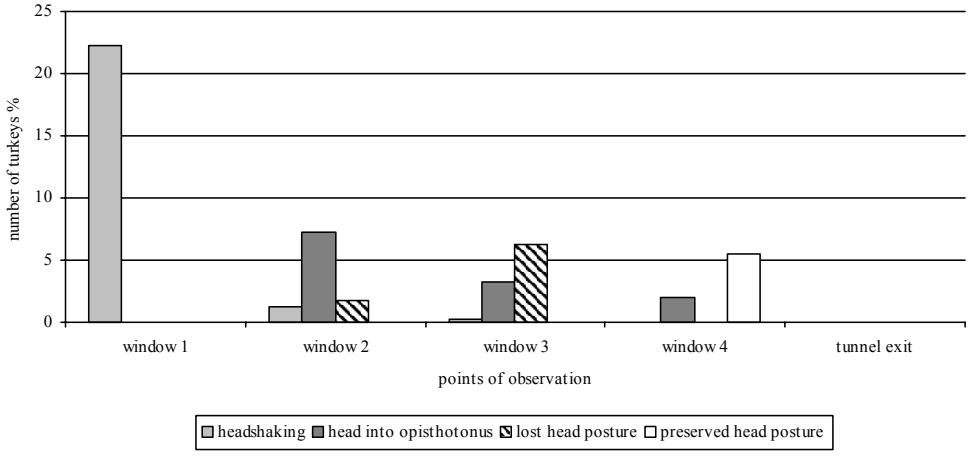

Figure 2. Head posture and reactions of turkeys during stunning with CO_2 at different positions in a gas tunnel in %. (n_1=2600)

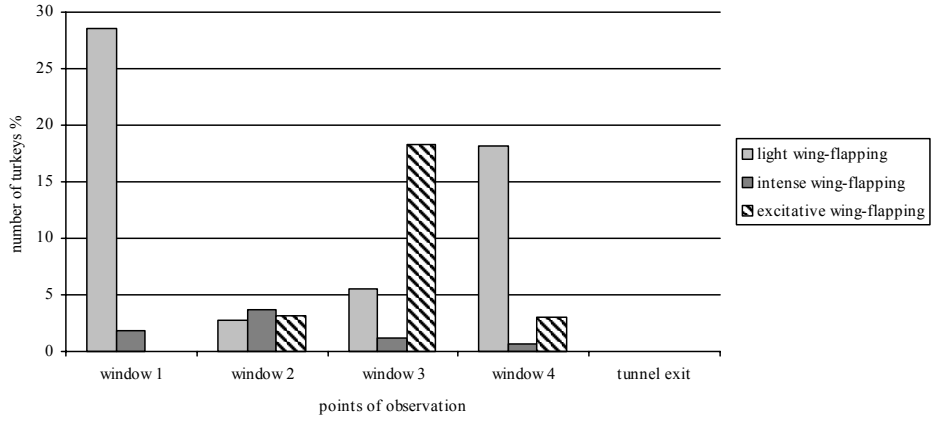

Figure 3. Wing-flapping of different intensity of turkeys during stunning with CO_2 at different positions in a gas tunnel in %. (n_1=2600)

Reflexes: Table 2 describes the results of the examined reflexes. Nearly 80 % of the birds had their eyes shut. The painful interphalangeal reflex could not be observed in any case. This indicates that the animals were obviously dead when leaving the stunning tunnel. This assumption was also supported by the fact that none of the animals which were kept aside for 3 to 4 minutes showed any sign of regaining consciousness again.

Table 2. Reflexes observed in turkeys (n_2=230) after CO_2-stunning

Reflex	Eyes open	Half-opened eyes	eyes closed
Eyelid closure	1 (0,4 %)	46 (20 %)	183 (79,6 %)
	No reaction		Positive reaction
Interphalangeal reflex	230 (100 %)	----------	0

DISCUSSION

This preliminary study shows that stunning of turkeys in the described V-shaped CO_2-tunnel results in an adequate anaesthesia at the end which allows an easy shackling of unconscious birds which also do not recover consciousness while bleeding to death. This is to be welcomed under aspects of animal welfare and occupational safety because it eliminates a lot of disadvantages of the electrical water bath method.

However, there are also some observations which give cause for concern. When entering the gas atmosphere many animals show signs of discomfort. They shake their heads, breath with opened beak and move their tongue. Nevertheless in general they stayed relatively calm which may be also due to the transport stress they suffered before and the narrowness in the crates. The light wing flapping and moves of their feet while entering the descending tunnel were probably for keeping balance.

The observation of window 1 presented all birds being conscious and registering the CO_2. They showed headshaking and wing flapping as defence moves because the gas irritated the mucosal membranes of their respiratory tract. This respiratory distress was rising in window 2. The birds felt breathless and showed intense wing flapping. By these gas concentrations unconsciousness started, heads moved into opisthotonus and finally lost their posture. Excitations in form of wing flapping started at the lower verge of the second window and mainly happened at window 3. Here many animals had already lost their head posture, only a few still showed opisthotonus and a few isolated birds showing headshaking or breathing deeply. It may not be excluded that some turkeys experienced this state of CO_2 concentration (ca. 70%) still being conscious. In window 4 the majority of turkeys reached the state of unconsciousness. The light and a few intense wing flaps can be interpreted as the end of the excitations. At the exit of the tunnel all birds lay down in the crates with lost posture and predominant closed eyes. The investigation of the reflexes seems to support this conclusion.

CONCLUSIONS

CO_2-stunning in this V-shaped gas tunnel seems to have a good potential to effectively stun turkeys at the end which allows a simplified shackling of unconscious birds which also do not recover consciousness while bleeding to death.

However, in the initial stunning phase the negative effects of CO_2 are observed such as breathlessness, head shaking and wing flapping indicating a high degree of discomfort, possibly the birds may even experience pain and anxiety. This time span comprises about 60 sec.

Alternative methods should be introduced for this initial stunning phase which may eliminate these disadvantages for the birds, e.g. using a gas like argon in a multi-phase system which causes anoxia instead of hypoxia.

Future work will include examination of stab blood for catecholamines (short term stress indicators) as well as for lactate and glucose as meat quality parameters, including carcase temperature, pH, water holding capacity and conductivity after one and after 24 hours.

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CAN CO_2 STUNNING MEET WELFARE OF SLAUGHTER PIGS?

Nowak, B.[1], von Mueffling, T.[1] and Hartung, J.[2]

[1] Institute for Food Quality and Food Safety; [2] Institute for Animal Hygiene, Animal Welfare and Behaviour of Farm Animals, University of Veterinary Medicine Hannover, Germany

SUMMARY

Stunning of pigs using CO_2 is not undisputed as to animal welfare aspects particularly because of too long stun-to-stick times. 460 pigs were subjected to six treatments: 80 vol% CO_2 for 70 s and for 100 s, stun-to-stick interval 25–35 s; the 100 s treatment also stun-to-stick time of 40–50 s; and the same treatments with 90 vol% CO_2 accordingly. All animals were tested for clinical reactions, catecholamines and lactate. Stunning (80% or 90% CO_2) was found to be acceptable for animal welfare only in combination with 100 s exposure times and stun-to-stick times of 25 to 35 s.

Keywords: carbon dioxide (CO_2), animal welfare, stunning, catecholamines, epinephrine, norepinephrine, lactate, corneal reflex

INTRODUCTION

Approximately 240 million pigs were slaughtered in the EU-25 countries in 2006, the most in Germany (49 million), and Spain (38 million) (Polet, 2006). An increasing number of abattoirs across Europe, especially the larger ones, are changing over from electric to stunning of slaughter pigs by carbon dioxide (CO_2). Generally, more pigs per hour can be slaughtered if CO_2 stunning facilities are used. Besides, it is said to result in a better meat quality when compared to electrical stunning. Both stunning methods are legally accepted. Even though the Scientific Committee has considered different scientific opinions (ScVC, 1997; EFSA 2004) on the slaughter and killing of animals since 1993, no basic amendments have yet been made of Directive 93/119/EC (Anonymous, 1993). Although this Directive permits stunning with an atmosphere containing at least 70% CO_2 by volume in air for a minimum of 70 s, the Scientific Committee (ScVC, 1997) has proposed a concentration of at least 80% CO_2, and this has been implemented in different national regulations (for example in Germany) since 1999 (Anonymous, 2004). However, after stunning with 80% CO_2 for at least 70 s animals still show signs of consciousness before exsanguinations. An Opinion Paper of the EFSA (2004) recommends a concentration of 70% to 80% CO_2 at the first stop in the stunning pit and 90% at the bottom in addition to exposure to the gas for more than 100 s. Anyway, not only the CO_2 concentration and the time of exposure to the gas are important factors for animal welfare, but also the time from not being exposed to the gas anymore until sticking of the animals. This stun-to-stick time is recommended (Anonymous, 2004) to be not longer than 30 s after the last stop in the atmosphere or 20 s after the animal is removed from the stunning chamber. In any case, sticking must be carried out before the animal regains consciousness (EFSA, 2004). Throughout Europe slaughter facilities become bigger and more pigs are put into each stunning gondola, especially since the time of exposure has been recommended to be as much as 100 s (EFSA, 2004), this often resulting in long stun-to-stick

intervals for some of the stunned pigs. As gas stunning normally is a reversible stunning method, animals not bleeding to death fast enough after tipping will regain consciousness during the further slaughter procedure.

The objective of the investigation was to determine the impact of different CO_2 stunning procedures and stun-to-stick intervals on animal welfare. In the end the aim was to see if the CO_2 stunning of slaughter pigs can meet animal welfare criteria, meaning in the following that slaughtered pigs are not subjected to a realisation of pain after CO_2 stunning.

MATERIALS AND METHODS

In a commercial abattoir 460 pigs were slaughtered with six different slaughtering methods: slaughter pigs were stunned with two different concentrations of CO_2 ($\geq$ 80% and $\geq$ 90% volume in air) for two different exposure times (70 and 100 s). Stun-to-stick intervals were between 25 to 35 s. Additionally, animals were investigated when stuck 40 to 50 s following the 100 s exposure to the CO_2 atmosphere. In each stunning treatment, two to three (100 s exposure: also four) pigs were brought into the gondola for CO_2 stunning. A one-gondola dip-lift system was used. The motor of the gondola transporter was set to reach the bottom of the pit in 22 s and to return to the ejection level 25 s after restarting there. The actual gas concentrations to which the animals were exposed were determined by an Advance Optima System® (Hartmann & Braun Analysetechnik, Frankfurt a.M., Germany) both inside the gondola at the pigs' nose level and on the bottom of the pit. The animals were shackled and hoisted before exsanguination.

In order to get an overview if the pigs were stunned successfully under each stunning treatment, the depth of unconsciousness was determined by testing clinical reflexes or reactions. Twenty-five to thirty-five seconds after the animals were tipped out of the gondola and immediately after sticking, each pig was tested for a reaction to a painful stimulus on the nasal septum, followed by a digital touch of the cornea and the eye lid (corneal and palpebral reflex), and the auscultation of the heart beat (~ 40 s after tipping, for a duration of 20 s). During the whole time of exsanguination a second person recorded directed movements of the animals. The movements were graded into three categories: 1, negligible (up to five running motions, single head movements); 2, moderate (continuous but moderate running motions, head movements); and 3, profound (massive running motions, recurring movements of the whole body).

Beside clinical reaction, parameters (catecholamines, lactate) in the blood plasma of the slaughtered pigs indicating the realisation of stress were tested. The first sticking blood from each stunned pig was collected into a 10 mL EDTA- tube for haematological use (1.6 mg EDTA/mL blood; Sarstedt, Nümbrecht, Germany) pre-prepared with 250 µL of a stabilising solution consisting of EGTA (ethylene glycol bis-aminoethyl ether N, N, N', N'-tetra acetic acid) and reduced glutathione (both Sigma Aldrich Chemie, Germany) in an aqueous solution of pH 7.0 to 7.5. At exsanguination, the prepared tube was filled with approximately 10 to 15 ml of blood. The collected blood was immediately put on ice and centrifuged upon arrival at the Institute for 10 min at 1,500 x g to extract the blood plasma. Approximately 1.5 ml of plasma was transferred two times to reaction tubes which were immediately frozen at –80°C until analysis. Quantitative detection of norepinephrine and epinephrine from the blood plasma was carried out in the neurobiological laboratories of the University of Göttingen with a high-performance liquid chromatographic (HPLC) method (Musso, Vergassola, Pende & Lotti, 1990) routinely used for human and animal plasma. Di-hydrobenzylamine (10 µL / 10 ng substance) was added to the samples (blood plasma) as an internal standard. After 25 sample measurements a reading with

control plasma standards was integrated in the test procedure. The quantitative detection of lactate (L(+) lactate) was carried out in an Auto-Analyser System® (Bran & Luebbe, Hamburg, Germany) with a continuous-flow method for indirect observation of the enzymatic reaction of $NADH + H^+$ (Stahlhut-Klipp, 1975).

All data was analyzed using the SAS program, version 9.1 (SAS Institute Inc., Cary, NC, USA, 2004). For all animals proportion scaled, quantitative variables (catecholamine values, lactate) and nominal scaled, qualitative variables (clinical reflexes etc.) were detected. The results of the variables of the individual animals of each slaughter day (only one treatment) were tested for significant differences. No statistical differences were calculated between the animals of one day and between animals of one treatment. Conclusively, all animals assessed to one and the same stunning method variation were combined into one group, resulting in six different treatment groups. Every group was subjected to a descriptive and explorative data analysis (Proc. MEANS), and all measurements were tested for Gaussian distribution, and subjected to the Shapiro-Wilk test (Proc. UNIVARIATE). Box-plots used for graphic representation of data were created with Sigmaplot for W!indows 9.01 (SysStat Software, Inc., Point Richmond, CA, USA, 2004). The Wilcoxon's two-sample test and the Kruskal-Wallis test were used for two group significance tests if measurements were non-parametric (Proc. NPAR1WAY), and the t-test if distribution was parametric[0] (Proc. T-TEST). The following levels of significance were defined: $p < 0.05$, significant; $p < 0.01$, highly significant; and $p < 0.001$, most significant[0].

RESULTS AND DISCUSSION

The results of the positive clinical reactions are given in Figure 1. The data on clinical parameters for evaluating the depth of unconsciousness were gathered immediately after hoisting and sticking. The figure gives the relative occurrence in percent of the positive clinical reactions of the animals for each stunning procedure. Overall the most positive answers to stimuli, visible movements or audible heart beats occurred in animals stunned with 80% CO2, especially GC8-70, followed by GC8-100 with a stun-to-stick interval of 40 to 50 s. The same distribution was found with 90% CO2 stunning, with the most positive findings in the GC9-70 procedure, followed by GC9-100/40-50s.

According to Holst (2001) and EFSA (2004) presence of a positive corneal/palpebral reflex in more than 5% of the animals is a sign of an unsuccessful stun. Furthermore, there should be no spontaneous blinking of the eye and convulsions whatsoever, and there should be only brief gagging and gasping. In light of these demands, stunning with 90% (88% to 91%) CO_2 for 100 s will result in successful stunning, whereas the time from stunning to sticking is of minor importance. Gasping and convulsions were observed in pigs especially frequently after stunning with 80% CO_2, most often after 70 s and 100 s exposure in combination with a stun-to-stick interval of 40 to 50 s. There also were high numbers of pigs gasping and with convulsions after stunning with 90% CO_2 for 70 s.

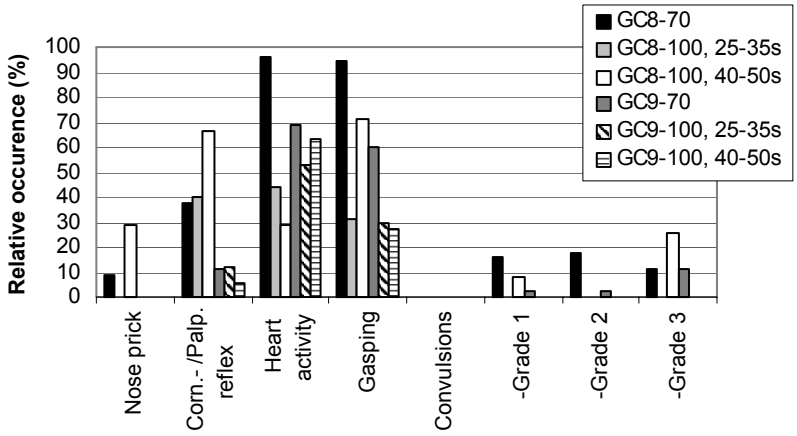

Figure 1. Percentage (relative occurrence) of positive clinical reactions under different stunning procedures: 80% CO_2 by volume in air: GC8; 90% volume in air: GC9, at different duration times in the stunning chamber (70 s, 100 s) and stun-to-stick intervals (25 to 35 s, 40 to 50 s)

Figure 2 shows the results obtained for epinephrine and norepinephrine in a box plot diagramme. There were no statistically significant ($p > 0.05$) differences in epinephrine or norenephrine levels when the animals were stunned with 80% or 90% CO_2 for 70 s or 100 s (GC8-70, GC8-100, etc.) and with stun-to-stick intervals of 25 to 35 or 40 to 50 s (GC8-100/25-35s GC8-100/40-50s, etc.). This was also due to high standard deviations. Total epinephrine values ranged from 149 nmol/L (with GC8-70) to 1721 nmol/L (with GC9-70). The lowest median epinephrine value was found in the GC8-70 experiment and the highest median value in the GC9-100/25-35s experiment. Norepinephrine levels ranged from 133 mmol/L to 3648 mmol/L (both with GC9-70). Norepinephrine median values were lowest with the GC8-100/40-50s procedure, and highest with the GC9-100/40-50s procedure. The ratio of norepinephrine (NEp) to epinephrine (Ep) ($F_{NEp:Ep}$) was about 2.7 for all stunning procedures tested here.

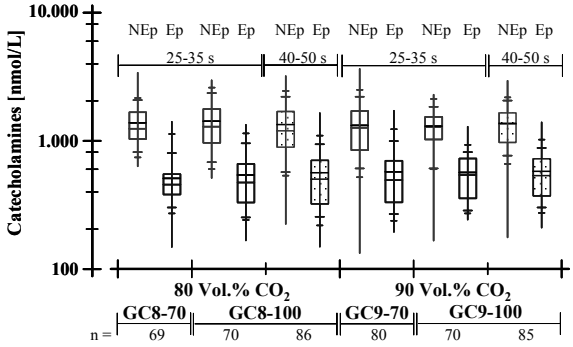

Figure 2. Effect of different CO_2 concentrations (80 vol%: GC8; 90vol%: GC9), different stunning times (70 s, 100 s), and stun-to-stick intervals (25 to 35 s, 40 to 50 s) on plasma concentrations of the catecholamines norepinephrine (NEp) and epinephrine (Ep). Box plot

Figure 3 shows the variation in and distribution of the lactate concentrations in the plasma of the slaughtered pigs. Overall values ranged between 2.41 mmol/L (GC9-70) and 35.8 mmol/L (GC8-70). Lactate levels in calm, rested pigs are said to vary from approximately 0.1 mmol/L blood plasma (Neubert, Gurtler, & Valentin, 1996) to about 2.0 mmol/L (Jensen-Waern & Nyberg, 1993). The broad range in all treatments shows that under every stunning method we seem to get stressed animals. However, the median lactate values after 90% CO_2 stunning were lowest with GC9-70 and highest with GC9-100/40-50s. Stunning methods with 80% CO_2 resulted in higher median lactate values: the lowest values with GC8-100/25-35s and the highest with GC8-70. The differences in lactate levels between all stunning procedures ranged from most significant ($p < 0.001$) to significant ($p < 0.05$). Extremes of over 30 mmol/L lactate were found after stunning for only 70 s both with 80% and 90% CO_2 (Figure 3). When the animals were kept in the atmosphere for 100 s, values were frequently above 20 mmol/L; the highest were 24 mmol/L, which was found with GC8-100/25-35s, and 21 mmol/L, with GC8-100/40-50s. The highest lactate level with the two GC9-100 procedures was 18 mmol/L.

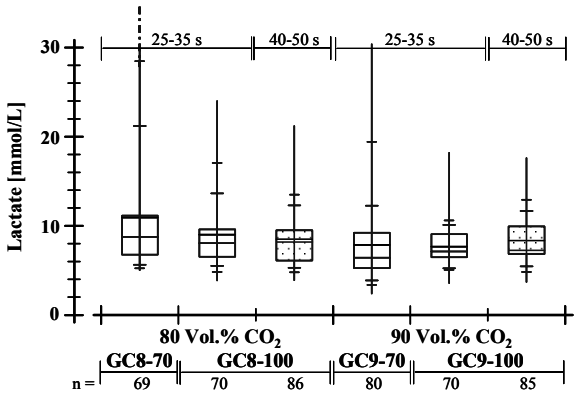

Figure 3. Effect of different CO_2 concentrations (80% by volume in air: 80 Vol.%, GC8; 90% volume in air: 90 Vol.%, GC9) at different stunning duration times (70 s, 100 s) and stun-to-stick intervals (25 to 35 s, 40 to 50 s) on plasma concentrations of lactate. Box plot

In conclusion our results of the testing of clinical parameters show that stunning with 80% CO_2 for 70 or 100 s correlated with high overall numbers of pigs with positive reflex reactions. A stun-to-stick interval of between 40 and 50 s after stunning in 80% CO_2 for 100 s in particular is not acceptable for animal welfare reasons; nor does exposure for 70 s in a stunning chamber with 90% CO_2 result in successful unconsciousness. These findings clearly show the importance of short stun-to-stick intervals for animal welfare. Therefore, the most important combination of factors to ensure sufficiently humane stunning has been said to be long exposure time to the gas (e.g. 150 to 160 s) with a stun-to-stick interval of about 70 to 90 s (EFSA, 2004; Holst, 2001). It has also been recommended that concentrations of 85% to 90% CO_2 be used, with exposure times of 100 to 120 s and stun-to-stick intervals of no more than 30 to 35 s, after exposure for 150 s, stun-to-stick intervals of 60 to 75 s may also be acceptable (EFSA, 2004; Holleben et al., 2002). This was confirmed by our results. Since new gas stunning systems place up to seven or eight pigs in each stunning unit, where stun-to-stick intervals of 70 to 80 s are not unusual (EFSA, 2004), it

is essential that action be quickly taken regarding new legal recommendations in light of these findings.

CONCLUSIONS

The presented results are not able to eliminate doubts if the presently applied CO_2 stunning techniques follow animal welfare. Particularly too long stun-to stick-intervals. Proposals are given with which the stress of the animals can be kept to a minimum within the framework of the existing legal regulations. What appears to be the best for the protection of the animals as well as safeguarding the meat quality is at present a combination of high CO_2 concentration (90 vol%), sufficiently long retention time in the gas atmosphere (100 seconds) and the implementing of the exsanguinations incision at the latest 20 seconds after emission from the stunning pit. Nevertheless, it still appears necessary to "shadow" carbon dioxide stunning critically both experimentally and in the practice in order to improve the protection of animals in this extremely sensitive socio-political area. Last but not least, this is one of the important veterinary tasks in the abattoir.

ACKNOWLEDGEMENT

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ROUTE PLANNING REDUCES THE COSTS OF ANIMAL TRANSPORTATION: ANIMAL WELFARE VERSUS ECONOMICS

Håkansson, N.[1], Henningsson, M.[2], Rönnqvist, M.[3] and Wennergren, U.[1]

[1] IFM, Theory and modelling, Linköpings Universitet, Sweden;
[2] MAI, Linköpings Universitet, Sweden; [3] NHH, Bergen, Norway

SUMMARY

Animals are more stressed on long transport routes with stops at many farms. The positions of farms and abattoirs are the basic properties that set the limits for route planning. Mobile abattoirs can reduce the cost of transportation and increase the welfare for the animals. The trade-offs between welfare and profit can be reduced by effective route planning. We have, by computer simulations, investigated how trade-offs differs between areas in Sweden and in general landscapes. The general results are applicable to any area and hence for animal transportation in general.

Keywords: animal transport, route optimization, 1/f noise, DFT, cost, animal welfare

INTRODUCTION

Long animal transports are both stressing for the animals and expensive. During transport, animal stress can be caused or increased by: weather conditions, handling, road conditions, meeting unfamiliar animals and long transport distances (Atkinson 2000). Longer transports could cause a higher percent of PSE (pale soft exudative), DFD (dark firm dry) and bruising defects on meat (Atkinson 2000).

We have studied how transport distance and the number of stops vary with respect to landscape structure and transport strategies. The basic structure of a landscape, distribution and number of farms and abattoirs, determines the limits for route optimization. We have tested different transport strategies in Sweden and specific areas in Sweden: Skåne, Östergötland and Västergötland. We also used neutral (virtual) landscapes with different landscape structures. By using virtual landscapes we can get a large data-set, analyze structures that are perhaps not available in our sample of the real world and get more general results.

Animal transport routes can be optimized with respect to animal welfare, economics or a combination of both. We have analyzed how the trade-offs between these changes with more effective route planning. We have made an algorithm that generates transports on a scale from very random to almost like the route planning algorithm of Clarke and Wright. Route planning for animal transports has been done in some areas in Sweden; for example in Uppsala (Ljungberg et al. 2006) and Västergötland (Algers et al. 2006). With our model of both optimized route planning and route planning that might resemble what is done today; we can compare the effects of effective route planning on a larger data set.

The number of abattoirs determines how good the routes can become in Sweden given that the farms are where they are. We have studied the effect of the small abattoirs and how transport distances could be shortened with mobile abattoirs.

We will see that the landscapes in Sweden have different basic properties for route planning, and what the gain is of effective route planning. We will also se how small-scale and mobile abattoirs influence the transport costs in Sweden.

THEORY AND METHOD

We have focused on models of cattle transport. We have used the coordinates for 22657 farms with cattle in Sweden. To analyze the differences in different regions we have chosen three areas of size 100x100 km, in Skåne, Östergötland and Västergötland, with 2517, 1021 and 2026 farms respectively. To analyze how transports in these landscapes differ we have placed 1–16 animals from a uniform distribution on each farm and simulated transports with different transport strategies. Sweden has 28 large and 40 small abattoirs, and we have assumed that animals are transported to the nearest of these abattoirs.

Transport strategies

The different transport strategies we use are: (i) Clarke and Wright is a heuristic which is commonly used in route planning and (ii) a modified version that more resembles a good choice than actual planning. Both algorithms starts with every farm on its own route and makes sure no route has more than 16 animals or is longer than 500km. Clarke and Wright iteratively connect the farms that will increase profit the most (minimize truck distance). The other method works in a more random way and resembles good choices made by transport operators. It takes a farm at random, connects the farm to up to two other farms, takes a new farm at random and works through all farms in this way. The "up to two" is partly because no farm can be connected to more than two farms and partly because a farm can keep the connection to the abattoir. When connecting the farms we use a 2-dimensional probability distribution to decide which sites (farms and the abattoir) the farm will be connected. The probability for connecting two possible sites is: $p=\exp(-d^b/k^b)$ where d is the distance between the sites. The parameter b determines kurtosis (peakedness) of the distribution; a low b gives a high kurtosis and farms that are very close are more probably connected than those further apart. The parameters k and b determines standard deviation and determines how far away farms could be connected. Large k and b gives very random transports; on the contrary low k and b means that only very close farms will be connected and these routes are more similar to Clarke and Wright. We have used standard deviations 10, 25 and 50 km and kurtosis 10/3 and 1.5. Uniform distribution has kurtosis 4/3 and an exponential distribution has kurtosis 10/3.

Virtual landscapes

We compare the transports in Sweden with transports in virtual landscapes. A landscape with aggregated farms may have different properties than one with more uniformly distributed farms. To generate landscapes with different levels of aggregation we use spectral methods. We generate a fractal 2-dimensional 1/f-noise, with discrete Fourier transformation (DFT) (Halley et al.2004, Keitt 2000). We start with a Gaussian white noise landscape, i.e. a 100x100 matrix L with random placed values drawn from a Gaussian distribution. We transform L with DFT and scale the

amplitudes with $|f|^{-\gamma/2}$. This gives a spectral density function proportional to $|f|^{-\gamma}$. The inverse transform of the scaled matrix is the aggregated landscape.

The parameter γ measures aggregation. A high γ means that we have a landscape where waves with low frequencies dominate, which is the same as an aggregated or red landscape. For γ=3.5 we get very aggregated farms and γ=0 gives uniformly distributed farms. We have used γ 0, 0.5, 1, 1.5, 2, 2.5, 3 and 3.5 in the analysis on virtual landscapes.

The DFT gives a landscape of continuous values, but farms are discrete. Because we can have no negative farms we scale the landscape by subtracting the lowest value. Then we normalize the landscape so that it sums to wanted number of farms. After that we determine what limit fraction shall be rounded up to get a landscape with the wanted number of farms. We then distribute the farms uniformly in the squares. We test with 500, 1000, 1500, 2000 and 2500 farms.

To find γ for the landscapes in Sweden we fit a line to a log-log plot of amplitude versus frequencies of the spectral density function. The slope of this line depends on parameter γ and on the density in the landscape. We have by simulation estimated a numeric function between γ, density and the slope of this line; with that we estimate γ. We have estimated γ for Skåne, Västergötland and Östergötland to 1.5, 1.2 and 1.0 respectively.

The abattoirs are placed randomly in the simulated landscapes. Skåne, Östergötland and Västergötland have 2, 4 and 8 abattoirs respectively. So in the virtual landscapes we test with 1–8 abattoirs. We consider the abattoirs as periodic. When we model mobile abattoirs in Sweden we iteratively consider the farms with more than 100 km to nearest abattoir, and add a mobile abattoir in the area where the total distance gain is the most.

RESULTS, DISCUSSION AND CONCLUSIONS

For animal health we look at how far the animals are transported and how many stops there are on their routes. For the costs we measure how far the truck is transported. Because we want to compare different landscapes with varying number of farms and animals we divide the cost with number of animals, so it is cost per animal.

To evaluate the direct effect of route planning we compare the generated routes with the scenario that every farm gets its own route. This is not a realistic scenario, but by comparing these scenarios we can evaluate the effect of the transport strategies. We define relative animal transport distances by mean transport distance for animals divided by the same for the scenario where they get their own route. In the same manner relative costs are costs divided by costs for the scenario where every farm got its own route.

Skåne has the best properties for animal transportation; animals are transported almost twice as far in Östergötland (fig 1).

In all landscapes, virtual and real, we can see that Clarke and Wright give the best transports both for animals and economics. When evaluating relative welfare against relative costs, figure 1, we can see that Clarke and Wright decreases costs the most (about 40%) without increasing transport distance for the animals very much (<5%). The mean number of stops for the animals is 1.78 (varying between 1.59 and 1.92) for Clarke and Wright compared to 1.72–1.73 for the ‘good choice’ heuristics (varying between 1.53 and 1.88). Clarke and Wright gives 6–30% lower costs and 2–24% shorter transport distances for the animals than the other heuristics; the lower values are for the smartest heuristic of the ‘good choice’. For the study in Uppsala optimization could save 18% (Ljungberg et al. 2006). The study in Västergötland found potential savings 14%–23%.

An ANOVA on the transports in the virtual landscapes shows that the most important, about 90% of M.S., for total transport distances and costs are the number of abattoirs, #A, i.e. for transport distances (#A 92% and heuristic 8%) for costs (#A 87% and heuristic 12%). For the relative costs and welfare the heuristic is most important (about 90%), i.e. relative transport distances (heuristic 92%, γ 4% and #A 3%) and for relative costs (heuristic 91%, #A 4% and γ 4%). The number of farms and the interactions always explain less than 1%.

In figure 2, we can se the differences in costs and welfare in Sweden when removing the small abattoirs or adding mobile ones. With effective route planning the animals are transported about 13 km shorter with small abattoirs and yet 2 km shorter with mobile abattoirs. More interesting than the effects on the mean is the effect for the 2.5% of the cattle that are transported longest distances. With the small abattoirs this is decreased from 140 to 90 km and with the mobile ones it is decreased to 70 km (this is for Clarke and Wright heuristic).

The conclusions are that an effective route planning decrease both costs and mean transport distance for animals, but slightly increase the number of stops. Landscapes in Sweden have different basic properties and therefore different limits for route planning. The small-scale abattoirs in Sweden are important for costs and animal welfare and especially for those animals transported the longest. Mobile abattoirs could improve the welfare even more.

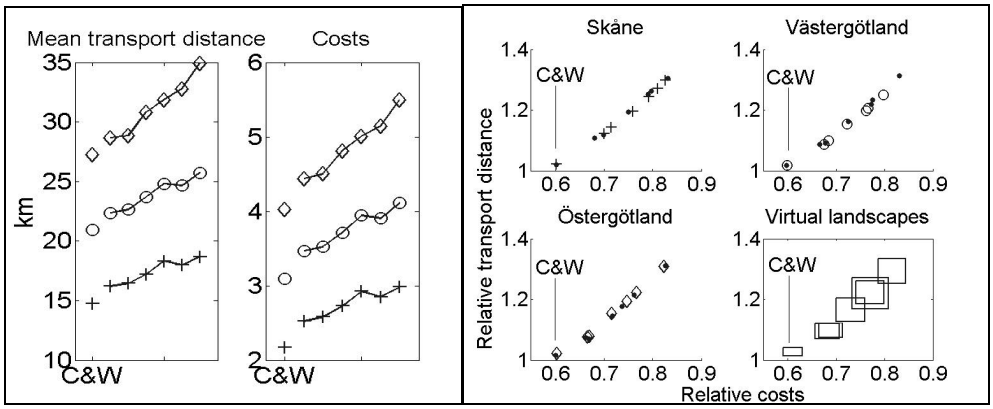

Figure 1. Plus signs is transport in Skåne, circles Västergötland, diamonds Östergötland and dots virtual landscapes. Clarke and Wright is indicated in the figures (C&W). The other markers are for the 'good choice' heuristic. Left: Mean over 20 replicats and redistributed animals for every replicate. The standard deviations are less than 0.6 km for the transport distances and less than 0.07 km for the costs. Right: Relative costs and mean transport distance in real and virtual landscapes. Mean over 10 replicats for virtual landscapes. Squares show 95% av the transport means per heuristic

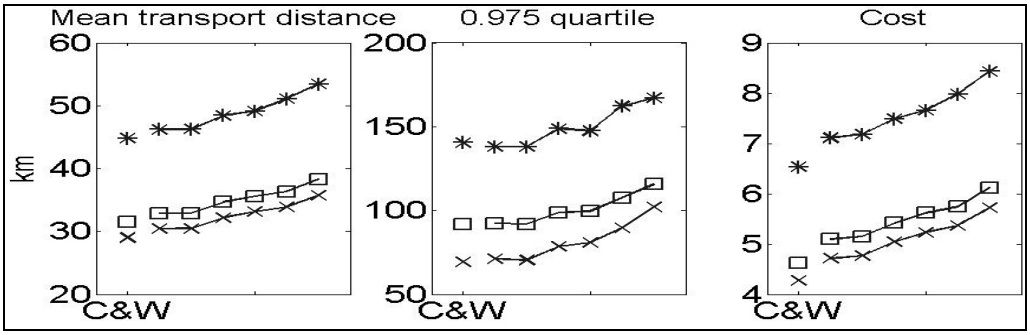

Figure 2. Transports in Sweden mean over 20 replicates. The standard deviations are smaller than 0.3 km, 1.1km and 0.03km. Stars are only large abattoirs, squares are small and large abattoirs and the crosses are large, small and mobile abattoirs

ACKNOWLEDGEMENT

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POSTER PRESENTATIONS

BLOOD GASES, METABOLIC PROFILE AND DEHYDRATION OF FEMALE AND BARROW PIGS TRANSPORTED FOR A PERIOD OF 7.5 HOURS IN MEXICO

Becerril-Herrera, M.[1], Alonso-Spilsbury, M.[2], Lemus-Flores, C.[3], Guerrero-Legarreta, I.[4], Olmos-Hernández, A.[2], Ramírez-Necoechea, R.[2] and Mota-Rojas, D.[2]

[1] Student from the Doctorate in Biological Sciences, Universidad Autónoma Metropolitana. Iztapalapa/Xochimilco. Calz. del Hueso 1100. Col Villa Quietud, México, D. F. 04960. Mexico; [2] Laboratorio de Etología, Producción Porcina y Fauna Silvestre. Dpto. de Producción Agrícola y Animal, Universidad Autónoma Metropolitana-Xochimilco. Calz. del Hueso 1100. Col Villa Quietud, México, D. F. 04960. Mexico; [3] Facultad de Medicina Veterinaria y Zootecnia, Universidad Autónoma de Nayarit; [4] Laboratorio Bioquímica de Macromoléculas. Dpto. de Biotecnología. Universidad Autónoma Metropolitana-Iztapalapa

ABSTRACT

The aim of the study was to evaluate the effect of gender on a series of blood gases, and dehydration in pigs transported for 7.5 hours. A total of 250 pigs (120 females and 130 barrows) were monitored and a blood sample was taken on arrival to the abattoir. Significant differences (P<0.05; Kruskal-Wallis) were observed for ear temperature, pCO_2, pO_2, K^+, Ca^{++}, lactate and haematocrit between genders. Females had greater values (P<0.01) than males for Ca^{++} (mmol/L): 1.48±0.02 *vs.* 1.42±0.01; lactate (mg/dL): 61.79±1.89 *vs*. 56.03±1.23, and ear temperature (°C): 39.51±0.07 *vs.* 39.12±0.07, respectively. Haematocrit was higher (P< 0.0001) in males (41.43±0.38% *vs*. 37.63±0.50%). Results show that males were more tolerant to transport stress than females.

Keywords: pigs, transportation, haematocrit, carcass pH, blood gases, lactate, abattoir

INTRODUCTION

Transportation is one of the most important stages in pigs handling before slaughter, since it influences meat quality and quantity (Gallo *et al.*, 2000); transport duration affects the welfare of transported pigs (Wajda & Denaburski, 2003) compromising physiological stress and/or physical fatigue. The aim of the study was to evaluate the effect of gender on blood gases, energetic profile, acid-base imbalance and dehydration in pigs transported over a 7.5 hours period.

MATERIALS AND METHODS

Animals

The study was carried out in a federal inspection plant from August to October, 2006. A total of 250 pigs (130 barrows and 120 females) Pietrain x (Yorkshire x Landrace) were studied. Transportation was done in livestock trailers with straw bedding, according to the animal care regulations in Mexico (Official Mexican Regulation, NOM-024-Z00, 1995). Pigs were transported for 7.5 hours without stops and were not fed, nor provided with water.

Rectal temperature at arrival

Rectal temperature was measured with a digital rectal thermometer immediately after unloading; each of the transported pigs was marked with a marker on the cervical region in order to identify them before slaughter.

Sacrifice and carcass traits

Pigs were sacrificed through electrical stunning. After stunning, 45 min post sacrifice, pH was measured in the hot carcass and after fast cooling in the cold carcass.

Blood sampling

Animals were monitored from slaughter to sale as cold carcass. A blood sample was taken from jugular vein within 15 seconds of restraining, and mixed with lithium heparin to impede blood gas alteration. In our experience, the blood sampling takes approximately 20 to 30 sec. In order to evaluate acid-base imbalance, energetic profile and dehydration, haematocrite (%), glucose (mg/dL), serum electrolytes [Na+, K+ and Ca2+ (mmol/L)] and blood lactate (mg/dL) levels, oxygen saturation [SaO2 (%)], and partial pressure of carbon dioxide [PaCO2 (mm Hg)] and oxygen [PaO2 (mm Hg)], were obtained by means of an automatic blood gas and electrolyte analyzer (GEM Premier 3000, Instrumentation Laboratory Diagnostics S.A. de C.V. México).

Statistical analysis

Results were analysed through a Kruskal-Wallis test.

RESULTS & DISCUSSION

Mean and standard error of the mean for traits monitored at arrival at the abattoir are shown in Table 1.

Significant differences were observed between genders ($P<0.05$) for ear temperature (ºC), pCO_2, pO_2, K^+, Ca^{++}, lactate and haematocrit, with regard to the transport effect and tolerance of gender to stress. Females presented a great level ($P<0.01$) of plasmatic Ca^{++} (mmol/L) and lactate (mg/dL), and higher ear temperature (°C) than males on arrival to the slaughterhouse.

Higher lactate concentration found in pigs transported over a short period (7.5 h), might be an indicator of physical stress and might be one of the reasons for the lower pH values obtained in both genders (Pérez *et al*., 2002).

There was a dramatic increase ($P< 0.0001$) in the haematocrit levels for the males (females 37.63±0.50, *vs*. males 41.43±0.38%), this may be due to a greater loss of body fluids since males deposit more fat and there is an inverse relationship between fat deposition and amount of liquids. There was a higher pH ($P <0.01$) in both hot and cold carcass for the castrated males compared with the females (Table 2). Gilts showed values in the normal pH range (5.8–6.2).

Table 1. Mean and standard error of the mean of the energetic metabolism, acid-base imbalance, and blood gases on arrival at the abattoir according to the gender of transported pigs for 7.5 hours

Traits	**Normal values***	**Females n = 120**	**Barrows n = 130**	Kruskal-Wallis test
		Mean ± SEM	**Mean ± SEM**	**P (α)**
Restraining time (sec)	–	21.55±0.47	22.03±0.47	0.4720
Ear temperature (ºC)	37.5–39	39.51±0.07	39.12±0.07	0.0003
Blood pH	7.33–7.45	7.37±0.01	7.36±0.01	0.7699
pCO_2 (mmHg)	40±2.3**	39.22±0.44	42.04±0.30	0.0001
pO_2 (mmHg)	71±3**	23.44±0.56	21.50±0.38	0.0051
Na^+ (mmol/L)	133–171	148.05±0.36	148.20±0.24	0.7206
K^+ (mmol/L)	4.5–6.5	4.93±0.04	5.51±0.05	0.0001
Ca^{++} (mmol/L)	2.4–3.0	1.48±0.02	1.42±0.01	0.0067
Glucose (mg/dL)	48–135	103.80±1.49	100.40±1.61	0.1241
Lactate (mg/dL)	0–10	61.79±1.89	56.03±1.23	0.0116
Haematocrit (%)	36–43	37.63±0.50	41.43±0.38	0.0001

*After Bollen *et al.* (2000), Plonait & Bickhardt (2001) and Tello (1991).
**From arterial blood.

Table 2. Mean and standard error of the mean for the carcass pH from transported females and barrows for 7.5 hours electrically stunned

Carcass traits	**Females n = 120**	**Barrows n = 130**	Kruskal-Wallis test
	Mean ± SEM	**Mean ± SEM**	**P (α)**
Hot carcass pH	6.45±0.01	6.52±0.01	0.0040*
Cold carcass pH	6.00±0.02	6.22±0.01	0.0001*

CONCLUSIONS

On the basis of the information derived from the present study, it can be concluded that contrary to expectations, males were more tolerant to transport stress than females, this is observed by the biophysical profile at arrival, but also in the hot and cold carcass´ pH values, which were significantly more acid in the females compared with the males.

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EMERGENCY KILLING OF POULTRY DURING DISEASE OUTBREAKS IN THE NORDIC COUNTRIES

Berg, C.

Swedish Animal Welfare Agency, PO Box 80, SE-532 21 Skara, Sweden

SUMMARY

This presentation summarizes methods available for killing poultry during outbreaks of epizootic or zoonotic diseases. Available methods for killing poultry are listed and discussed with relation to practical aspects, biosecurity, animal welfare, occupational hazards and other pros and cons of each method. The stunning methods mentioned are blow to the head, electrical stunning and captive bold stunning, all mainly applicable for small or possibly medium-sized farms. The killing methods discussed are bleeding, neck dislocation, maceration, injection of barbiturates, carbon dioxide (in-house, in containers, in flow-containers), nitrogen and argon gas, of which some are suitable also for large poultry flocks.

Keywords: animal welfare, biosecurity, carbon dioxide, epizootic, euthanasia, gas killing, stunning

OBJECTIVE

This presentation aims at summarizing the various methods available for killing poultry during outbreaks of epizootic or zoonotic diseases, such as Avian Influenza. It is generally acknowledged that from a worldwide perspective, animal welfare has often been severely compromised during efforts to control such disease outbreaks, although there are methods available which could have been considered both efficient from a disease control perspective and acceptable from an animal welfare perspective.

METHODS

This presentation is based on a document produced after a Pan-Nordic meeting on Avian Influenza (AI) in Copenhagen 2006. It covers different options regarding the killing methods that may be used during a possible future outbreak of AI or other epizootic diseases in poultry in the Nordic countries. The document is based on scientific knowledge and practical experiences, both from the Nordic countries and from other parts of the world. The content of the EG Directive 93/119 on the Slaughter and Killing of Animals has been taken into account, as has the Scientific Report of the Scientific Panel for Animal Health and Welfare on a request from the Commission related to welfare aspects of animal stunning and killing methods (Question N° EFSA-Q-2003-093), the Scientific Opinion on Animal Health and Welfare aspects of Avian Influenza (EFSA-Q-2004-075) and the OIE Terrestrial Animal Health Code 2005. The EG Directive 93/119 lists a number of methods which can be used for killing (Annex C), but leaves the field relatively open

for other methods to be used for disease control purposes (Annex E), as long as they adhere to the basic requirements to spare the animals any avoidable excitement, pain or suffering during the process.

RESULTS

Bisecurity

When killing birds during a disease outbreak the main objective is efficient disease control, i.e. to prevent further spreading of the disease in question. Both infected and non-infected flocks may be killed in the process of trying to stop further spreading of an infection. From this, it is obvious that a very important factor when choosing a killing method is its efficiency from a disease control and biosecurity perspective. It must be easily and rapidly available, and the capacity must be large enough to cover all poultry holdings in an infected region and surrounding areas in a relatively short time. However, other factors must also be taken into account.

Animal welfare

Animal welfare consequences must be considered, and the method chosen should involve as little stress, pain or suffering as possible. Basically, one should strive for the same level of animal welfare during emergency killings as during planned killings or standard slaughter. This means that a killing method should either cause immediate death, or sedation followed by death, or death in already stunned/unconscious animals. In general, it should involve as little manual handling of the birds as possible, as birds in large commercial flocks are not used to manual handling and may perceive catching and handling as very stressful.

Workers' safety

Furthermore, operators' safety is a crucial aspect. The method chosen must be safe for the staff involved in carrying out the culling, both with respect to not involving unnecessary risks itself, and by not risking spreading the infection to humans, if it is a zoonosis.

Practical considerations to be made

From a practical point of view, a number of other aspects will also influence the choice of killing method. Such factors are: Type of birds – domestic poultry or wild birds? Species – chickens, turkeys, ducks…? Flock size – Ten birds or 100 000 birds at one site? Age – day olds or adult birds? Body weight – is it possible to carry out manual procedures? Housing system – is it possible to catch the birds? Can the house be sealed?

Contingency plans and training of staff

Whichever method chosen, it is important that the staff involved is well trained for their task and skilled in carrying it out. Contingency plans and basic training should preferably involve technical aspects (how to carry out the different procedures from a technical point of view), practical aspects (back-up, logistics, etc.), animal welfare aspects and also psychological aspects (killing and handling large numbers of dead birds is often very mentally tiring). Poorly trained staff is a risk factor for unsuccessful flock killings, resulting in anything from human frustration and anger or poor animal welfare to inefficient disease control or even disease spreading. It is advisable

always to have an experienced veterinarian present during the entire process. Vets are often also involved in taking blood samples or other types of samples from the birds when the flock is killed. During a larger outbreak of an epizootic disease it can be difficult to find the necessary number of vets with substantial poultry experience. A plan for rapidly training vets who are normally engaged in general farm animal practice should therefore be considered. The applied decision process will have to be well established before an outbreak is even diagnosed, in order to work in the field during an outbreak.

Stunning: Blow to the head

This method is rapid and efficient when carried out properly. Proper practical training and instruction of staff is crucial. However, the method is not suitable for larger flocks, as it is time consuming, tiring and requires large staff, and should preferably only be used at small farms. After stunning, the birds can be killed by neck dislocation or bleeding.

Stunning: Electrical stunning

Small, handheld electrical stunners are commercially available. They are used for small scale poultry slaughter and are relatively easy to use on all poultry species. Such stunners can also easily be mounted on a wall to facilitate rapid stunning of larger numbers of birds. These stunners are suitable for small and mid-size farms.

In some countries, mobile containers incorporating standard electrical water-bath stunners for killing poultry are available. In this case, the stunning is immediately followed by an automatic neck cutter for killing by bleeding. Such mobile containers can kill a relatively high number of birds and may therefore be suitable for mid-size farms, but they can be difficult to clean and disinfect, and transporting them between farms might be considered a biosecurity risk. Using a low frequency and high current setting will lead to cardiac arrest in the birds, and the method can then be considered to be a stun-to-kill method, which eliminates the need for bleeding.

Stunning: Captive-bolt stunning

Captive-bolt guns designed specifically for poultry are commercially available. Depending on what species is involved, a flat or a cone-shaped bolt head can be used. Under non-emergency conditions, the use of a captive-bolt gun should be followed by killing using another method, such as neck dislocation or bleeding. However, the damage to the skull caused by the bolt is normally enough to cause the immediate death of the bird. In an emergency killing situation, omitting the bleeding procedure can therefore be considered.

Killing: Bleeding

Death by a rapid bleed out after the severing of both carotid arteries is the standard procedure during slaughter of poultry, and can of course also be used for emergency killing, unless it is found to be inappropriate from a biosecurity perspective, which is often the case.

Killing: Neck dislocation

Neck dislocation is a method often used by poultry farmers to kill sick or injured birds during the production period. Neck dislocation should be carried out by rapidly stretching and twisting the neck, in order to achieve a separation of the vertebrae and a rupture of the blood vessels. If the dislocation occurs in the upper part of the neck, between the skull and the first cervical vertebra,

there may be damage in the lower parts of the brain, causing immediate unconsciousness and death. However, if the dislocation occurs further down, there is a risk that immediate unconsciousness is not achieved. Because of this, birds should be stunned before killing by neck dislocation is carried out. For very small chicks, approximately under the age of 2 weeks, neck dislocation can be carried out in an acceptable way without prior stunning. When killing hens, broilers and other birds with a body weight up to approximately 3 kg (5 kg if staff is experienced and well trained), neck dislocation can usually be carried out manually without any special equipment, which is one of the advantages of the method. For larger birds, like geese, it is often necessary to use mechanical aids. The method is suitable for small flocks only, as the work is quite strenuous, but can sometimes also be used for mid-sized flocks when other options are not available. Neck dislocation is not an aesthetically pleasing method.

Killing: Maceration (Instantaneous Mechanical Destruction, IMD)

Maceration, which is done by placing chicks in a homogenizer with a rapidly rotating knife, is commonly used for killing malformed chicks or surplus male layer chicks at hatcheries. If carried out correctly, the method leads to instant death in the chickens. It can be used during a disease outbreak for killing day-old chicks at hatcheries.

Killing: Barbiturates

Killing birds by injecting a barbiturate solution (sodium pentobarbital) is very time consuming. A very rapid effect is seen when injecting the fluid intravenously, but this procedure requires skilled personnel. When injecting the fluid intra-abdominally some birds may react, as the substance is irritating, and it has been reported that it takes quite some time before the birds die from the injection. An injection given, by mistake, into the air sacks is another possible bird welfare problem, as it is painful and inefficient. In any case, killing by injecting barbiturates is only recommended for very small groups of poultry, or when other methods cannot be used, e.g. due to aspects of blood or tissue sampling for later diagnostics or other analyses. For geese and ducks however, there are only a limited variety of alternatives, and the method can, under certain conditions, be considered a method of choice for these species. Also other types of drugs used in veterinary medicine can be considered for the euthanasia of small numbers of birds.

Killing: Carbon dioxide (CO_2)

CO_2 is a gas which is commercially used to stun broilers and turkeys prior to slaughter. Depending on the concentration of the gas and duration of the exposure, the gas is lethal. CO_2 is a heavy gas that has been shown to be aversive to birds, especially at higher concentrations. Although the method is debated, it is currently commercially used and widely accepted, especially in disease outbreak situations. To avoid some of the aversion, methods have been developed under which the birds are first exposed to a lower concentration of CO_2 to make them unconscious, and after that to a higher concentration (approx 80%), as aversion is not a problem in already unconscious birds. This is technique is used, for example, when broilers are stunned with CO_2 in slaughter plants. If the duration (exposure time) is long enough, it has been reported that birds will die at considerably lower concentrations, like 40%. Nevertheless, some researchers recommend high concentrations, especially when using CO_2 in poultry houses where the process is less well controlled. By using high concentrations of the gas, losses due to poor sealing and problems related to uneven distribution of the gas inside the house can be compensated for to a

certain extent. From an animal welfare point of view it is essential that all birds are killed rapidly, and it can be argued that high concentrations should be used, to be on the safe side.

The use of CO_2 on waterfowl, such as geese and ducks, is controversial. There are reports showing that the method is effective, but several field reports indicate that the time taken to achieve unconsciousness and death is considerably longer in these species than in hens and turkeys, which raises questions from an animal welfare point of view. If possible, other methods should be used.

CO_2 at hatcheries

CO_2 killing is commercially used for killing half-hatched, malformed chicks or male layer chicks at hatcheries. The chicks are placed directly into a small container with a high concentration of CO_2. The method can be used during a disease outbreak for killing day-old (i.e. < 72 h) chicks at hatcheries.

CO_2 in small containers medium containers or large plastic bags

In some countries, spent laying hens are killed by being put into a relatively small or medium sized CO_2 container, a wheelie bin, which is pulled through the aisles between the rows of cages. The CO_2 concentration is relatively high (50% or higher), and only a limited number of birds are placed into the bin at a time. As soon as one layer of birds appears to be dead, a new layer of birds are placed on top of them, and so on until the container is full. The dead birds are then emptied from the bin, and the procedure starts all over again. The method can be questioned from an animal welfare point of view due to the direct exposure to high CO_2 concentrations, and also from an ethical point of view. In an emergency situation however, the tolerance for this would probably be higher than when killing spent hens. An advantage of this system is that the direct exposure to high CO_2 concentrations will result in very rapid loss of consciousness in the birds, compared to, for example, in-house gassing. The method only requires a small amount of CO_2, and the equipment is inexpensive. Compared to in-house gassing, an advantage is that the birds will not be exposed to cold gas, and neither will there be a problem with noise. One disadvantage is that the birds have to be caught before killing, and the system can mainly be considered suitable for small to mid-size farms. It may also be considered for cage farms, where in-house gassing can be difficult. If the containers are to be moved between farms, thorough cleaning and disinfection will be necessary. Other types of containers have also been developed, for example the mid-size steel container system which has a capacity of 25 tonnes, or commercially available systems with double-layer plastic bags. These system works in the same way as the wheelie-bin system, except for the fact that the containers are placed outside the poultry house, and the birds are carried manually to the containers. Because of this these systems can mainly be considered suitable for small to mid-size farms.

CO_2 in flow containers

A Danish egg producer-owned company has constructed a CO_2 container for killing spent hens, designed to improve the killing rate and the animal welfare aspects of this process. The hens are placed into the container directly on a conveyor belt, taking them from a lower concentration of CO_2 to a higher, thereby killing the birds. After this, another conveyor belt brings the birds out of the gas container again, and into a macerator. The resulting pulp can then easily be evacuated into a closed bulk transport vehicle and transported away. According to the company the system has a

capacity of approximately 4000 hens an hour during killing of spent hens, which makes it suitable for mid-size to large farms.

Whole-house CO_2 gas killing

Whole-house killing with CO_2 is used in some countries as an alternative to the slaughtering of spent hens, especially in remote areas or when flocks are deemed unfit for transport or consumption. It has also been used in some European countries during recent AI outbreaks. The method is generally considered acceptable when used in a disease control situation, although it should be recognized that it has several drawbacks from an animal welfare point of view. When dealing with poultry diseases that pose a danger to human health, the hazards related to catching live birds inside poultry houses must be considered before a decision on the method for killing the birds is made. The method is only suitable if the poultry houses are designed in a way which allows them to be reasonably sealed, although it is not necessary to achieve complete closing up. The main advantage of the method is that there is no need to catch the birds before killing. This speeds up the process, especially on large farms, and also decreases the risks of exposing humans to whatever infection prompted the killing. The method is relatively quick; there are studies indicating a time frame of approximately 10–15 minutes from the start of the procedure until no hens show signs of consciousness. This, however, will depend on the size of the house and the number of gas inlets.

Killing: Nitrogen

Nitrogen is an inert gas; it's odourless and tasteless, does not induce a sense of breathlessness and does thus not cause aversive reactions in the birds. It can, if mixed with CO_2, be used for killing poultry in containers. Trials have also been carried out using liquid nitrogen for the in-house killing of laying hens in battery cage systems.

Killing: Argon

Argon is an inert, heavy, non-explosive gas, which can be used to kill poultry and other animals. In some countries, argon is used for stunning (and in reality also killing) poultry at slaughter plants. Argon is considered an animal welfare friendly gas, as it is odourless and does not cause aversive reactions. It has been shown to work well both for hens, turkey, ducks and geese. It is also considered relatively safe to handle. However, the gas and the necessary equipment are quite expensive and therefore not considered suitable for in-house killing. Argon can be mixed with CO_2 (80%/20%) for container use and might be used for on-farm killing. Such trials have been carried out, mainly in the UK. This system is referred to as the 'containerised gassing unit' (CGU), and is basically a steel chamber in which two sets of standard transport modules with birds are placed before the gas is fed into the CGU, after which death follows very rapidly.

Killing: Carbon monoxide (CO)

Using pure carbon monoxide to kill poultry is regarded as an efficient method. However, it has been questioned if the method is acceptable from an animal welfare point of view, as it has been reported to cause convulsions before the onset of unconsciousness. Usually, the birds are then placed in containers with CO. The primary reason for not using CO is the occupational hazard, as CO is lethal at rather low concentrations and carries a risk of explosion, thus constituting a considerable health and safety risk for the staff involved.

Killing: Shotgun

Using a shotgun to kill birds is not generally recommended. Domestic birds should instead be possible to catch or keep inside a building or a pen. Semi-wild birds reared in enclosures and later released but fed by humans in a limited area may be killed by shooting, but only if catching is unsuccessful. It can be considered contra-indicated to hunt wild birds using a shotgun if a contagious disease is thought to be present in a specific area – it may actually scare and scatter the possibly infected birds over a larger area, thereby increasing the risk of spreading the disease.

Killing: Alphachloralose

The feeding of the lethal substance alphachloralose can be used to facilitate the catching of semi-wild birds in enclosures. As the method often causes unconsciousness but not death, death should always be confirmed using another, more reliable method, and therefore the method is not generally recommended.

Other methods

There is currently research being undertaken on developing a type of foam, similar to fire-fighting foam, for in-house killing of poultry, but until further details of the substances involved and their effects on the birds are known, and reliable reports on bird welfare aspects, operators' safety aspects and biosecurity aspects are available, the method cannot be generally recommended.

Cyanide gas, which is extremely toxic, has previously been a widespread method for in-house poultry killing, but due to questions related to animal welfare and obvious aspects of workers' safety it is no longer recommended when other alternatives are available.

There are several methods which are generally not considered acceptable, mainly for animal welfare reasons but also because some of them would not be optimal from a biosecurity / disease control point of view. Therefore, the following methods should be avoided under any circumstances: Placing live birds in plastic bags and burning them; using impure carbon monoxide (exhaust fumes) for gassing, any type of blunt trauma except a blow to the head for a limited number of birds; drowning; suffocation; and decapitation of non-stunned birds.

CONCLUSIONS

It is concluded that a variety of methods for on-farm killing of poultry during disease outbreaks are available, and that several of them can be considered acceptable from an animal welfare point of view. It is also concluded that a separate decision regarding the method of choice will have to be made for each farm in each case of disease. Furthermore, it is concluded that it is essential to have contingency plans elaborating on these aspects beforehand, as the decision process will have to be very rapid once an outbreak has been diagnosed.

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INDUCED RESPONSE OF SOME WELFARE INDICATORS IN SLAUGHTERED SHEEP

Pârvu, M. [1], Andronie, I.[1], Andronie V.[2] and Dinu, C.[3]

[1] Animal Production Departments, [2] Internal Medicine Department, [3] The Physiology and Ethology Department, Faculty of Veterinary Medicine "Spiru Haret", Bucharest, Romania

SUMMARY

Our research has monitored how stressful inside slaughter handling, stunning and immediate slaughtering proves to be to the sheep in comparison to their transport as such.

Sheep within slaughterhouse environment have responded to stressful factors such as new spaces, unfamiliar handling personnel, other animals' presence and unknown smells by exhibiting an increase to the maximum in metabolic activity, attention alert and a change in behaviour. The physiological responses induced by these factors have been emphasized by an increase in the respiratory rate as well as variations in plasmatic cortisol, haematocrit and glucose levels in different moments of the study. The results revealed the fact that electric stunning and slaughtering were more stressful than their manhandling in the slaughterhouse.

Keywords: sheep welfare, stress, slaughter

INTRODUCTION

According to the valid legislation, the conditions that must be provided for animals, which are to be slaughtered in the period previous to their slaughtering touch, transport means: loading and unloading, transport, feeding and watering before slaughtering, animal handling in the slaughterhouse and stunning. Sheep handling during transport is widely acknowledged as a stressful manoeuvre especially at loading and unloading times onto and out of transport means. Animal movement down the handling lanes may be just as stressful as it is not conducted gently. The manner in which animals to be slaughtered is handled during transport and the waiting period prior to their slaughtering is characteristic of the ethical standards men display in connection with animals.

An appropriate stunning method and bleeding time in the slaughterhouse decrease animal stress. This fact was underlined by Gregory et al. (1984), who, followed research states that electric stunning at head level in sheep is reversible if slaughtering does not happen immediately and their welfare is poor. Sheep stress prior to stunning is also emphasized by Anil et al. that following his research in 1996 states: sheep that witness other animals' stunned are much more stressed than the latter, data confirmed by our research.

MATERIALS AND METHODS

Research was conducted on 18 adult sheep (n: 18, of Țurcană and Țigaie breed), male and female with an average weight of 47 kg, divided into groups A and B which were moved from the waiting paddock to slaughterhouse on the handling lane in order to be stunned and then slaughtered. The slaughtering interval between the groups was 30 minutes during which the group B sheep were stationed in the waiting paddock.

The sheep included in the study were identified, clinically examined, and each had an intravenous catheter inserted, which was kept until after stunning. All manoeuvres were completed two hours prior to the stunning and measurements were taken in the waiting box, handling lane, immediately after stunning and after slaughtering. Blood samples were taken in the waiting box, on the handling lane prior to stunning, immediately after that and 3 minutes after bleeding. The blood samples were then analyzed in the laboratory and the variation in plasmatic cortisol level (by means of RIA method), haematocrit (by means of the electronically method ABCVet) glucose (by means of a portable glucometer).

Sheep were introduced in the waiting box by ten and then were weighed and handled one by one towards the stunning area. The stunning was carried out by means of an electric stunner placed behind the sheep's ears, after which the animals were hooked and bled for 15 –20 seconds. The heart rate was measured with a Polar monitor in the waiting box, prior to stunning and immediately after bleeding.

RESULTS AND DISCUSSION

The fact that metabolic stress during transport is lengthy was also confirmed by Knowles et al. (1995) and stated in our previous research. This is due to the emotional stress, deprivation of fodder and water and last but not least animal movement. Man-handling of lambs during the first 10 days of their life studied by Markowitz et al. (1998), resulted in their getting used to the people as they grew up. Moreover, sheep are able to distinguish between their caretakers, who have a calming effect on them at stressful times, and strangers, aspect shown by Boivin et al. (1997).

The abnormal reactivity of the heart rate is characteristic of the chronic stress responses, by autonomous nervous system stimulation (Wiepkema and Koolhaas 1993).

In the sheep studied, the heart rate values showed a variable evolution (figure 1) from the waiting box until after the bleeding period with the first group monitored (A). Since these animals had already been used to handling during transport, this manoeuvre was no longer regarded as stressful inside the slaughterhouse. The stress was signalled only prior to stunning time on the handling lane towards the stunning box. As far as the second group (B) was concerned, where the time until stunned was 30 minutes, the heart rate recorded higher values only in the waiting box due to the sheep agitation on the handling lane, moment after which the values were similar to those of the first group until after the slaughtering. There was no obvious tachycardia recorded such as the case of their transport, especially when loading and uploading.

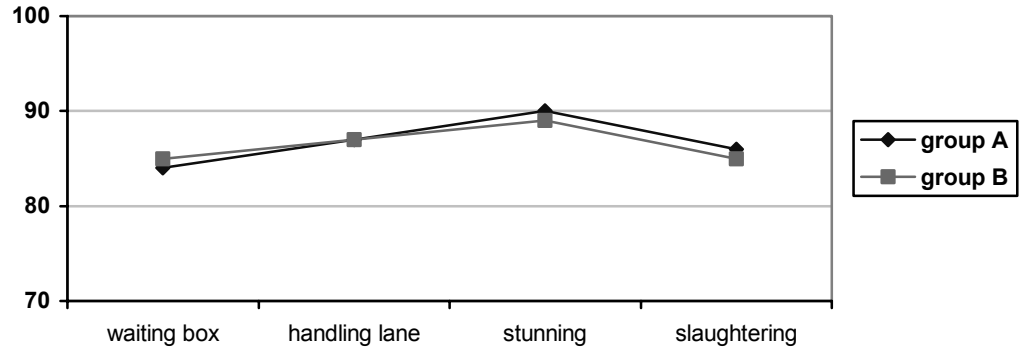

Figure 1. Heart rate evolution (bpm) recorded in sheep (n: 18) during their handling inside the slaughterhouse

The level of biochemical indicators in animal welfare varied at analyzed times, enabling us to evaluate sheep stress inside the slaughterhouse.

By studying the responses to various stress factors in adult sheep we were able to point out that if we take a sheep out of the flock, the emotional condition created will lead to a slight increase in the haematocrit. In the case at hand the haematocrit recorded a higher increase during stunning and bleeding than the sheep handling moment (figure 2) for group A, whereas for those in group B the level was lower during waiting time after which had a significant rise during stunning and slaughtering. This was due to the effect the cathecolamine induce on the spleen under stress. There is a rise in sympathetic – adrenergic activity that stimulates the spleen contraction.

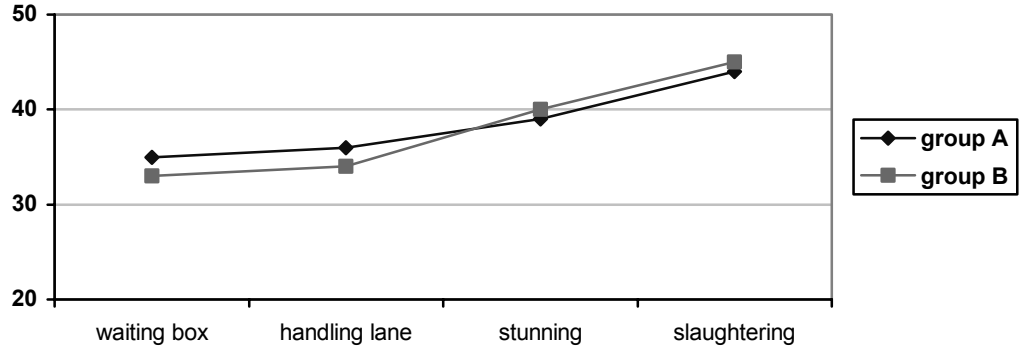

Figure 2. The hematocrit level (%) recorded in sheep (n: 18) monitored by this study

Propionate is one of the volatile fatty acids produced in the rumen and it is transported to the liver and turned into glucose and oxaloacetate; part of the metabolic energy will be obtained by oxidation of the acetate while another part will be deposited in glucose. This will be later used as an energy source until the existing hepatic glycogen stock is exhausted.

The level of glucose in our research recorded slight variations (figure 3) while staying within the species limits in the waiting box and the handling lane, both for sheep in group A, and those in group B. During stunning and slaughtering, the sheep exposed to stress factor responded by an increase in plasmatic glucose levels as a result of the cathecolamine action.

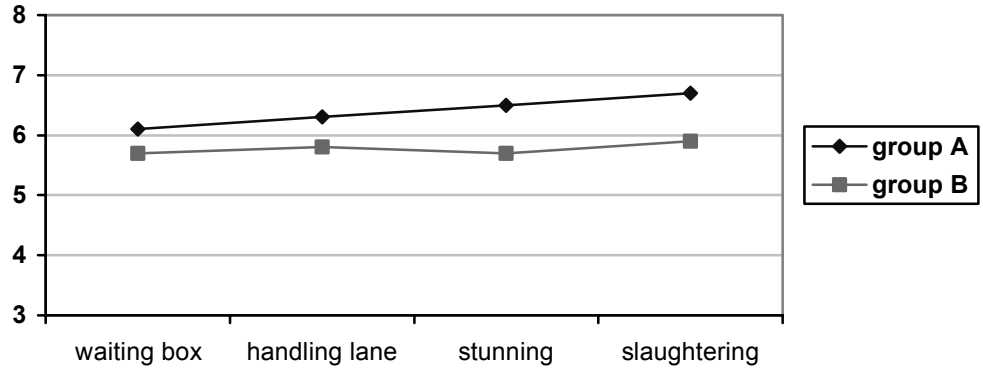

Figure 3. The glucose level (mmol/l) recorded in sheep (n: 18) monitored by this study

Another welfare indicator monitored in our study was the plasmatic cortisol whose level was higher than normal for this species during the three monitored moments for both groups (picture 4). There was a significant increase in the sheep that were first stunned and then bled. This was due to the response to the stress stimulus during electric stunning as a result of the direct stimulation of the neuroendocrine nervous system.

The stress increases the adrenal glands' ability to produce glucocorticoids which results in a stronger response to acute stressful factors in chronically stressed animals (Jensen et al.1996; Terlouw et al. 1997). HPA axis responds to long-term stress in sheep, which involves changes in control systems such as plasmatic cortisol level. This may recover or drop below pre-stress levels simultaneously with an increased pituitary-corticoadrenal response to additional physiological stress factors.

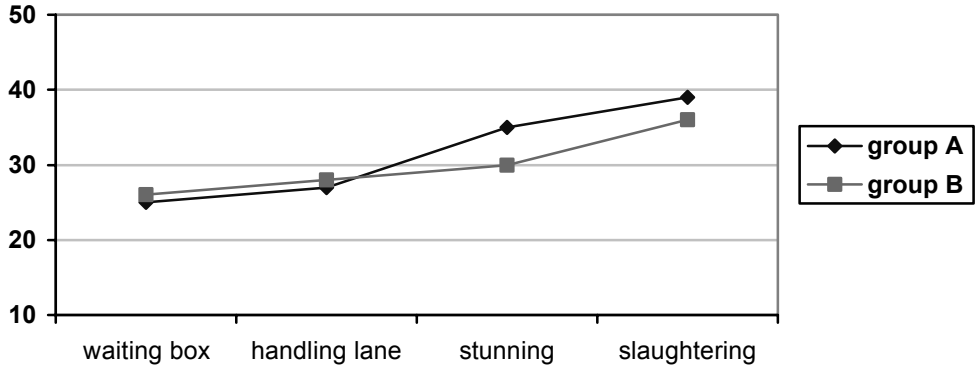

Figure 4. The plasmatic cortisol level (μg/dl) recorded in sheep (n: 18) monitored by this study

CONCLUSIONS

The increase in some welfare indicators monitored during certain situations was due to the sheep's vocal communication through alarm calling destined to alert the congeners. Thus the increase of the heart rate in sheep that spent longer in the waiting box could be due to these alert callings but also to the agitation following the handling and weighing manoeuvres of the animals destined to be stunned.

Although we did not set out to monitor the sheep's conduct, they reacted vocally both in the waiting box and on the handling lane and some of the animals even showed fear before stunning.

Our research has also shown that sheep handling inside the slaughterhouse is a manoeuvre less stressful than their transport, once the animals are already used to these operations. The most important stressful factor consisted of the sheep electrical stunning and slaughtering, which was indicated by the constant rise in biochemical indicators and plasmatic cortisol levels.

The time spent by the sheep in the waiting box until stunning did not influence the variation in indicators monitored at the stunning and slaughtering time. In addition, sheep that were stunned at a 30-minute interval did not display different responses to stress factors from the former even if they witnessed some of the manoeuvres.

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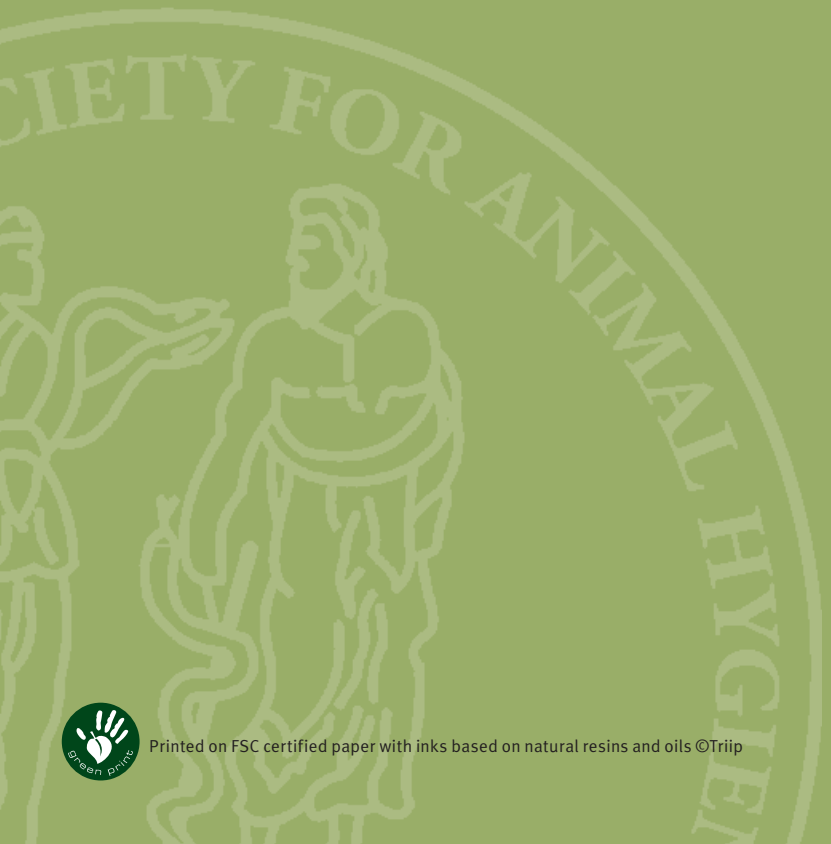

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