EFFECTS OF THE INTRODUCTION OF LACTIC ACID BACTERIA IN THE FEED OF BROILERS: FROM THE FARM TO THE PROCESSING PLANT

Chemaly, M.[1], Postollec, G.[2], Maurice, R.[3], Boscher, E.[1], Houdayer, C.[1], Labbé, A.[1], Hervé, G.[1], Gentilhomme, G.[2], Boilletot, E.[4], Brézillon, C.[4], Quéré, F.[4], Salmon, F.[4], Fravalo, P.[1] and Burel, C.[2]

[1] UHQPAP, [2] UAA, [3] SEAC: AFSSA, BP53, 22440 Ploufragan;
[4] PRIMEX, La Gare de Baud, BP 21 56440 Languidic

SUMMARY

This trial aims to assess the effect of a feed complement (*Lactobacillus* spp.) in broilers in order to find an alternative to the antibiotic growth promoters. The trial was performed in the AFSSA experimental husbandry where the animal performances were recorded regularly, up to the slaughterhouse. At the rearing level, sampling included faecal content, while at the processing level, samples from neck and thigh skins were taken. Coliforms, *C. perfringens*, *Enterococcus*, *S. aureus*, *Pseudomonas* and Lactic acid bacteria were enumerated in these samples. The results showed a significant improvement in daily weight gain (P=0,049) in the batch receiving the lactic acid strain at 10^5 cfu/ml. At this concentration, the product showed a positive effect (P=0,031) on the health of the birds. Regarding the digestive flora, no significant effect was recorded in the samples. The product did not modify the bacterial count of the treated batches nor at the rearing neither at the processing level.

Keywords: alternative to AGP, digestive flora, lactic acid bacteria, poultry production

INTRODUCTION

The ban of antimicrobial growth promoters (AGP) from poultry feed has led to a decline in animal health status, an arise in digestive troubles (Puterflam et al., 2007) and the use of therapeutic drugs (Grave et al., 2004). In turn, this could affect the colonisation of the animal intestinal tract by opportunistic bacteria and/or pathogens, leading to a degradation of the hygienic quality of animal products meant for human consumption. Moreover, the microbiological quality of processed poultry meat is directly linked to the flock contamination via the slaughtering process and the contamination of carcasses mainly during evisceration. In study aiming to assess the risk of *Salmonella* contamination of turkey carcasses, it was shown that the risk of meat contamination at the slaughterhouse and cutting plant is associated with the carriage rate in live animals: a group with a low carriage rate represents a moderate risk (0 to 4% contaminated meat), while one with a high carriage rate represents a higher risk (11 to 40% contaminated meat) (Petton *et al*., 2003). There is therefore an increasing need to find alternatives to the use of AGP efficient in reducing the risk of animal diseases, improving the balance of the gut microflora and allowing reducing pathogen shedding. In this regard, a previous study aimed to screen different "natural" products led to the selection of a lactic acid bacteria based product regarding its efficiency to reduce the shedding of *Salmonella* by young turkeys (Petton et al., 2005).

The aim of this study is to test the effect of this lactic acid bacteria based product regarding the growth performances and the digestive flora equilibrium balance during the rearing and the influence on the microbiological quality of the carcasses at the processing level.

MATERIAL AND METHODS

Animals

5000 "Ross" broilers were divided into 15 batches distributed over 3 experimental treatments. Each treatment was replicated five times with 250 animals in each floor pen leading to a stocking density of 16 animals/m^2.

Lactic complement

The lyophilised lactic acid bacterial strain was added to the drinking water. The birds of the treatment 1 (T1) received 10^5 cfu/ml, and those of the treatment 2 (T2) received 10^6 cfu/ml, while in the control treatment (T0); the animals did not receive the product.

Animal performances

Animal growth performances were recorded by weighing a sample of 30 animals by floor pen at 7, 21 and 36 days. Feed intake and feed efficiency of each floor pen were estimated from the difference between the total quantity of feed distributed and the weight of the remaining feed at 7, 21 and 36 days.

Microbiological analyses

For the study of the digestive flora, 15 animals from each treatment (3 per floor pen) were euthanized for faecal content sampling in order to follow the amount of total coliforms, *C. perfringens*, *Enterococcus* and Lactic acid bacteria at the rearing level. At the processing plant level, 15 samples of each treatment were taken from neck and thigh skins. On these samples, thermophilic coliforms, *S. aureus*, *Pseudomonas* and Lactic acid bacteria were enumerated the day of slaughter. After 7 days chilling at 4°C *Pseudomonas* and Lactic acid bacteria were enumerated on neck skins.

Statistical analyses

The effects of the feed complement were tested using when possible the parametric test Anova followed by Tukey's test. When the conditions of normality and homogeneity of the variances are not completed, the non parametric test of Kruskall-Wallis was performed followed by Mann and Whitney's test in case of significant differences between the treatments

RESULTS

Growth performances

The administration of the lactic product did not show any significant effect ($p=0.698$) between 0 and 7 days on the daily weight gain (DWG): the control and treated animals presented a DWG around 19 g/animal/day (table 1). Between 7 and 21 days, animals treated with 10^5 cfu/ml showed a significant higher DWG ($p=0.090$) than the other animals. Despite the lack of statistical

signification, treated animals with 10^6 cfu/ml had a higher DWG than the control ones (table 1). During the last rearing period, between 21 and 36 days, all the animals receiving or not the product had a similar DWG: about 88 g/animal/day. Finally, the lactic product had a significant beneficial effect (p=0.049) on the final body weight of the animals, but only at the dose of 10^5 cfu/ml: 2255g for T1, while 2235g for T2 and 2216g for T0.

Table 1. Animal daily weight gain (DWG) expressed in g/animal/day

Treatments	**0–7 days**	**7–21 days**	**21–36 days**
T0 (control)	19.5 ± 0.4	51.5 ± 1.6 (a)	87.9 ± 2.4
T1 (10^5 cfu/ml)	19.1 ± 1.0	53.6 ± 1.1 (b)	88.6 ± 1.5
T2 (10^6 cfu/ml)	19.2 ± 0.5	52.7 ± 1.0 (ab)	88.1 ± 2.5
	NS (p=0.698)	**S** (p=0.090)	**NS** (p=0.932)

Reported values (mean ± standard deviation; n=5) have been statistically analysed using the test of Kruskall-Wallis followed by Mann and Whitney's test. S: significant (p<0.05); NS: non significant (p>0.05)

The treatment by the lactic product did not significantly affect either the feed gain ratio (FGR) (table 2), nor the feed consumption.

Table 2. Animals feed gain ratio (FGR)

Treatments	**0–7 days**	**7–21 days**	**21–36 days**
T0 (control)	1.15 ± 0.03	1.46 ± 0.03	1.79 ± 0.09
T1 (10^5 cfu/ml)	1.17 ± 0.03	1.41 ± 0.04	1.76 ± 0.08
T2 (10^6 cfu/ml)	1.15 ± 0.04	1.44 ± 0.01	1.78 ± 0.05
	NS (p=0.756)	**NS** (p=0.137)	**NS** (p=0.779)

Reported values (mean ± standard deviation; n=5) have been statistically analysed using the test of Kruskall-Wallis followed by Mann and Whitney's test. S: significant (p<0.05); NS: non significant (p>0.05)

Health status

In general, no major health problem did occur during the rearing period except a short term diarrhoea which appeared at the beginning of the rearing. A non typable *Escherichia coli* was found in the faeces in the same period. A visual analysis has been realised over 50 chicks per floor pen based on the distinction between spoiled animals from clean ones in order to estimate the number of animals which contracted the diarrhoea (table 3). The statistical analysis showed that the number of animals treated by 10^5 cfu/ml was significantly lower (p=0.031) than the number of animals from the other treatments (table 3).

Table 3. Diarrhoea observed on chicks at the beginning of the rearing period

Treatments	**Diarrhoea (% spoiled chicks)**
T0 (control)	22 ± 13 (a)
T1 (10^5 cfu/ml)	8 ± 8 (b)
T2 (10^6 cfu/ml)	22 ± 8 (a)
	S (p=0.031)

Reported values (mean ± standard deviation; n=5) have been statistically analysed using the test of Kruskall-Wallis followed by Mann and Whitney's test. S: significant (p<0.05) ; NS: non significant (p>0.05)

MICROBIOLOGICAL ANALYSIS

At the rearing level

The treatment by the lactic product did not affect the bacterial count of lactic acid bacteria, total coliforms, *Enterococcus* and *C. perfringens* as no significant differences have been observed between control and treated animals at any sampling period (table 4). However, there is a significant effect of the rearing period on the means of bacterial count. In general, for all the microorganisms studied, the lowest count was noted between 7 and 21 days of rearing (table 4).

Table 4. The means of bacterial count in animal faeces (log (10) cfu/g).

Microorganisms	Treatments	0–7 days	7–21 days	21–36 days
Lactic acid bacteria	T0 (control)	9.2 ± 0.6	7.7 ± 0.5	9.3 ± 0.3
	T1 (10^5 cfu/ml)	9.2 ± 0.6	7.7 ± 0.6	9.2 ± 0.4
	T2 (10^6 cfu/ml)	9.1 ± 0.4	7.9 ± 0.4	9.3 ± 0.6
		P=0.739		
Total coliforms*	T0 (control)	8.4 ± 0.4	7.5 ± 0.6	7.9 ± 0.6
	T1 (10^5 cfu/ml)	8.0 ± 0.4	7.6 ± 0.6	7.8 ± 0.7
	T2 (10^6 cfu/ml)	8.0 ± 0.5	7.6 ± 0.6	7.8 ± 0.6
		P=0.501		
Enterococcus spp.	T0 (control)	8.4 ± 0.4	7.5 ± 0.6	7.9 ± 0.6
	T1 (10^5 cfu/ml)	8.0 ± 0.4	7.6 ± 0.6	7.8 ± 0.7
	T2 (10^6 cfu/ml)	8.0 ± 0.5	7.6 ± 0.6	7.8 ± 0.6
		P=0.746		
C. perfringens	T0 (control)	4.2 ± 1.9	3.2 ± 1.2	5.0 ± 1.5
	T1 (10^5 cfu/ml)	4.2 ± 0.5	2.5 ± 1.7	2.9 ± 2.1
	T2 (10^6 cfu/ml)	4.0 ± 1.2	2.2 ± 1.0	4.1 ± 1.9
		P=0.057		

Reported values (mean ± standard deviation; n=15) have been statistically analysed using the test of Kruskall-Wallis or the *Anova where the conditions of normality and homogeneity of variances were achieved. S: significant ($p<0.05$) ; NS: non significant ($p>0.05$)

At the processing level

The animals from our experimental rearing have been slaughtered at the beginning of the process in order to avoid cross contamination with animals coming from other farms. The day of slaughtering (d36), the lactic product, had no effect on the counts of the microorganisms studied on neck skins taken from control and treated animals (table 5). The mean counts of lactic acid bacteria, *Pseudomonas* and thermophilic coliforms were 5, 3.7 and 4 log(10) respectively.

Table 5. The means of bacterial count on neck skins (log(10) cfu/g) the day of the slaughtering (d36).

Treatments	Lactic acid bacteria	*Pseudomonas spp.*	Thermophilic coliforms
T0 (control)	5,1 ± 0,4	3,8 ± 0,4	4,4 ± 0,8
T1 (10^5 cfu/ml)	5,1 ± 0,3	3,8 ± 0,3	4,3 ± 0,6
T2 (10^6 cfu/ml)	5,1 ± 0,3	3,7 ± 0,5	4,2 ± 0,5
	p=0.997	p=0.947	p=0.755

Reported values (mean ± standard deviation; n=15) have been statistically analysed using the test of Anova

The same analysis was done on thigh skins taken from control and treated animals (table 6). No significant effect of the lactic product has been recorded except for the count of thermophilic coliforms (table 6); however, from a biological point of view, the difference between the concentrations was considered not significant. The mean counts of lactic acid bacteria, *Pseudomonas*, *S. aureus* and thermophilic coliforms were around 4, 3, 2 and 3 log(10) respectively. The comparison of bacterial counts on neck and thigh skins showed a higher level on neck skins for the same microorganisms (tables 5 and 6).

Table 6. The means of bacterial count on thigh skins (log(10) cfu/g) the day of the slaughtering (d36)

Treatments	**Lactic acid bacteria**	***Pseudomonas spp.***	***S. aureus***	**Thermophilic coliforms**
T0 (control)	4.9 ± 0.3	3.3 ± 0.6	2.2 ± 0.3	3.9 ± 0.4
T1 (10^5 cfu/ml)	4.6 ± 0.3	3.1 ± 0.5	2.3 ± 0.3	3.7 ± 0.3
T2 (10^6 cfu/ml)	4.9 ± 0.3	3.3 ± 0.3	2.4 ± 0.4	4.4 ± 0.4
	P=0.180	P=0.197	P=0.266	P=0.000

The samples from thigh skins have been chilled at 4°C for 7 days (d43) in order to check the effect of the lactic product during the storage on the level of lactic acid bacteria and *Pseudomonas*. No significant effect of the lactic product was observed on the counts of lactic acid bacteria and *Pseudomonas* (table 8). At d43, the level of lactic acid bacteria decreased by about 0.5 log(10) compared to the level at d36 (tables 6 and 7). Nevertheless, the average count of *Pseudomonas* increased significantly from 3log(10) at d36 to 7log(10) at d43 (tables 6 and 7).

Table 8. The means of bacterial count on thigh skins (log (10) cfu/g) 7 days after chilling at 4°C (d43).

Treatments	**Lactic acid bacteria**	***Pseudomonas spp.***
T0 (control)	4.3 ± 0.4	6.9 ± 0.4
T1 (10^5 cfu/ml)	4.4 ± 0.32	6.6 ± 0.5
T2 (10^6 cfu/ml)	4.5 ± 0.2	7.2 ± 0.4
	P=0.468	P=0.146

DISCUSSION

The growth performance of the chicken showed a significant effect, the lactic product added to the drink water at the dose of 10^5 ufc/ml (T1) compared to the control. However, no significant beneficial effect on the growth performance was observed at the dose of 10^6 ufc/ml (T2). The drinking water seemed yellowish at this higher dose, and it is likely that this high bacterial concentration altered the water quality, acting in turn on the intestinal flora of the birds and so on their growth performance.

If the lactic product at the dose of 10^5 ufc/ml improves the growth of the chickens, it seems that it improves also their health status. Indeed, even if a single opportunistic visual observation was made, it seems that the intake of this dose of the lactic product protected the birds against a short-time diarrhoea likely caused by a strain of E. coli at the beginning of the rearing period. The

visual observation indicated a lower proportion of animals affected by the diarrhoea and this protection could be related to the keeping of the balance of the intestinal flora.

The treatment had no effect on the studied digestive flora counts (lactic acid bacteria, total coliforms, *Enterococcus* and *C. perfringens*). In a same way, no significant effect between control and treated animals has been observed during the rearing period. The decrease in bacterial count at the period 7–21 days could be due to the changes in feed composition corresponding to feed transition periods.

At the processing level, the day of the slaughtering, the product had no effect on the level of the studied microorganisms (lactic acid bacteria, thermophilic coliforms, *Pseudomonas* and *S. aureus*) nor on neck skins neither on thigh skins as the comparison between control and treated animals showed no significant differences. During the storage period (7 days at 4°C) the product did not indirectly inhibit the growth of *Pseudomonas* on thigh skins as the level of these microorganisms was higher than the level the day of the slaughtering.

We conclude therefore that the lactic acid based product did not disturb the digestive flora tested during this trial and had no effect on the microorganisms recovered on neck and thigh skins at the processing levels. The benefit on *Salmonella* carriage reduction at the end of the rearing period in conventional herd condition will be performed.

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