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

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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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VERORDNUNG (EG) Nr. 1774/2002 des Europäischen Parlaments und des Rates vom 3. Oktober 2002 mit Hygienevorschriften für nicht für den menschlichen Verzehr bestimmte tierische Nebenprodukte, zuletzt geändert durch Verordnung (EG) Nr. 808/2003 der Kommission vom 12. Mai 2003 (EG-VO Tierische Nebenprodukte)