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DISINFECTION WITH METHYL BROMIDE OF POULTRY FEED AND STRAW LITTER CONTAMINATED ARTIFICIALLY WITH *Salmonella typhimurium*

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ABSTRACT

Experiments were carried out in a section of a poultry house having a volume of 6,000 m^3 to evaluate methyl bromide (MB) efficiency in the sterilization of feed and litter to prevent salmonella infection in poultry. Two concentration-time products (Ct product) of 1,500 and 2,000 $g.h/m^3$ were applied to five samples of crushed straw litter and poultry feed at 18°C and relative humidities (r.h.) ranging from 64 - 100%. The samples were contaminated artificially with *Salmonella typhimurium* (rifampicine-resistant) at rates of 5 x 10^2 - 10^6 colony forming units per gram (cfu/g). The higher Ct product gave 100% sterilization of all samples, but the lower Ct product gave complete sterilization only on the samples contaminated at a rate of 5 x 10^2 cfu/g.

INTRODUCTION

International interest in avian salmonellosis has been stimulated by public demand for foods of the highest quality. The poultry industry must take measures to eliminate salmonella infections. According to specialists on avian salmonellosis, perhaps 75% of the chicken and turkey populations in the USA are infected with one or more salmonella serotypes at some stage of their life (Snoeyenbos, 1977). Rigby *et al.* (1981) found *Salmonella typhimurium* to be the most common salmonella isolated from quarantined imported birds in Canada, and discussed the possible public health significance of exotic salmonella serotypes introduced by exotic birds. Over the past few years, there has been a dramatic increase in human cases of *S. enteritidis* in the USA, Canada, and Europe.

Benson and Williams (1978) found that *S. typhimurium* survived for at least 18 months at 11°C in both feed and litter, whereas at 25°C it survived for 16 months in feed and for 18 months in litter.

Gordon and Tucker (1965) reported that poultry kept on wire floors could be infected by one salmonella organism per gram of food, and it would be expected that much lower concentrations could cause infection in birds kept on deep litter where a continual reinfection from the litter occurs.

The complete elimination of *S. typhimurium* must involve:

- decontamination of the poultry house and poultry feed in order to preserve poultry health and production quality.
- decontamination of infected faeces and litter to prevent bacterial pollution of the environment.

At present, poultry houses are decontaminated by cleaning, and formaldehyde fumigation, followed by introduction of new birds a few weeks later (Samberg *et al.*, 1966) . This method takes time and is not really effective, partly because formaldehyde gas does not penetrate effectively deeper than about 2 cm. At present no solution has been proposed for the decontamination of feed and litter.

In other laboratory studies, Harry (1972) and Harry *et al.*, (1973) determined the degree of activity of methyl bromide (MB) against *S. typhimurium* in artificially-contaminated faeces and litter in small-scale tests. They found that concentration-time products (Ct products) of 800 - 1,600 g.h/m^3 would be required to eradicate this organism from poultry litter under optimal conditions.

Therefore, it was considered of interest to investigate the practicality of MB fumigation in a poultry house and to determine, under local climatic conditions, the gas concentrations required and the degree of disinfection obtainable. The preliminary experiment described below gave encouraging results that will require further study.

MATERIALS AND METHODS

Fumigation enclosure and system of measurement

The experiment was carried out in a 6,000 m^3 section of a poultry house in western France in September, 1991. Temperatures in the poultry house during fumigation ranged from 17 - 21°C, and the weather was windy and rainy. Given the poor weather conditions, it was deemed necessary to improve the seal of the poultry house section using a 30µm-thick polyethylene film with a permeability to MB of 2,000 gMB/24h/m^2.

Despite these measures, it was difficult to maintain the gas concentration at a constant level and, therefore, more gas was added 4 times during the fumigation period. When a Ct product of 1,500 g.h/m^3 was obtained, half of the contaminated samples were removed from the poultry house and the room was then sealed.

The liquid fumigant was released from a pressurized cylinder and applied at a dose of 80 g/m^3. Concentrations of MB were measured by

withdrawing samples and passing them through a thermal conductivity meter (Gas Master of Gow Mac and Fumiscope of R.K. Hassler).

Feed, litter and faeces samples were removed at the end of fumigation when a Ct product of 2,000 g.h/m^3 was obtained. For each MB introduction, a 3,000 m^3/h capacity fan was allowed to run for 15 min. For gas sampling, four tubes were placed at the following locations:

Tube 1 : in open container (samples of feed, litter, and faeces)
Tube 2 : in open middle container (samples of feed, litter, and faeces)
Tube 3 : in a bag of 25 kg feed contaminated by 25 g infected feed
Tube 4 : in the free space

Artificial contamination of 30 samples

The feed consisted of mash intended for "safe pathogen free" (SPF) layers. The litter consisted of crushed wheat straw. The moisture content (m.c.) of feed, litter, and faecal samples were on an average comparable to those found usually in French poultry houses.

Each sample weighed 25 g and was contaminated with a *S. typhimurium* rifampicine-resistant (rR) strain taken from an overnight broth culture. The levels of contamination are indicated in Table 1.

Salmonella content of treated samples

The presence of salmonella was investigated by :

- pre-enrichment (in buffered peptone water)
- enrichment (in tetrathionate broth with novobiocin and brilliant green solution and in Rappaport Vassiliadis broth RV 10)
- streaking on agar plate containing 0.1g/l rifampicine
- biochemical identification of strains growing on agar plates
- serotyping of salmonella by rapid slide test.

RESULTS

Penetration of Methyl Bromide

The results indicate that at temperatures ranging from 17°2 - 21°C, MB penetrated satisfactorily through brown wrapping paper bags and to the centre of a paper bag containing 25 kg poultry mash feed. Three hours after the treatment, the concentration in the 25 kg feed bags was equivalent to that in the free space (Table 2).

Bacteriological counts

The bacteriological results are given in Table 3.

Control Sample. After 48 h storage at 18°C: In the "wetter" faeces at 70% m.c., a 10 fold decrease was observed in the salmonella count as compared with the initial level of contamination. In the wet faeces at 50% m.c., proliferation of salmonella colonies to a level of 10^5 was observed in the

samples contaminated initially at levels of 10^2, 10^4, and 10^6 cfu/g. In feed and litter, the counts of rR *S. typhimurium* revealed only a few colonies in each sample (< 10^2 cfu/g).

Table 1 : Experimental conditions.

		Number of Samples		
		Ct methyl bromide (g.h/m^3)		
	Rate of Contamination (cfu/g)	Control	1,500	2,000
Faeces (70 % m.c.)	5.5 x 10^6	1	1	1
	5.5 x 10^4	1	1	1
Faeces (50 % m.c.)	5.5 x 10^6	1	1	1
	5.5 x 10^4	1	1	1
	5.5 x 10^2	1	1	1
Feed 1 (25 g) 12% m.c. in a double sealed paper (1) bag	5.5 x 10^3	1	1	1
Feed 2 (25 g) 12 % m.c. in a simple sealed paper bag buried in a 25 kg feed paper bag	5.5 x 10^3	1	1	1
Dry litter (15% m.c.)				
25 g	5.5 x 10^6	1	1	1
25 g	5.5 x 10^4	1	1	1
25 g	5.5 x 10^2	1	1	1
Duration		48 h	21 h	27 h
Temperature		18°C	Average: 18°C min: 17°C max: 21°C	

Samples fumigated at Ct products 1,500 g.h/m^3

In the wet faeces (50 % m.c.), even though control samples showed a contamination level of 10^5 cfu/g, *S. typhimurium* was not isolated from samples contaminated initially at 10^2 and 10^4 cfu/g. *S. typhimurium* was detected at a very low level in the sample contaminated initially at 10^6 cfu/g.

In the wetter faeces (70 % m.c.), no salmonella were isolated in the less contaminated sample, but 5.2 x 10^3 cfu/g were isolated in the sample contaminated initially at 10^6 cfu/g.

Table 2: MB concentrations during fumigation and Ct products (g.h/m^3) obtained.

Test No.	Time (h)	MB concentration (g/m^3) Tube 1 Feed 1 litter & faeces	Tube 2 Feed 1 litter & faeces	Tube 3 Feed 2	Tube 4 In free space	MB added (kg)
1	2	44	105	96	115	
2	3	141	98	96	99	
3	5	79	72	64	65	
4	5.5	81	85	84	94	2
5	8.5	81	81	77	76	
6	9.5	62	62	58	55	
7	12.8	51	48	47	45	
8	13	79	81	78	81	4
9	16.5	64	67	66	64	
10	17.3	79	87	83	83	4
11	19.5	79	77	73	68	
12	21	68	68	68	60	
Ct 21 h		1502	1540	1473	1499	
13	23.5	0	91	87	91	4
14	26.8	0	68	65	60	
15	27	0	65	60	57	
Ct 27 h			2015	1930	1948	

In both cases, the MB treatment demonstrated very satisfactory efficacy in the wet samples contaminated initially at levels of 10^2, and 10^4 cfu/g.

rR *S. typhimurium* was isolated (<10^2) in every sample of Feed 1 and Feed 2 (12% m.c.) and litter (15% m.c.) contaminated artificially with 5.5 x 10^3-10^6 cfu/g. In an adverse environment (dry), that does not favour growth of salmonella, the cells seem more resistant to MB.

Samples fumigated at Ct Products 2,000 g.h/m^3.

No rR *S. typhimurium* were isolated from any sample of feed and litter after treatment, irrespective of the initial level of contamination. Similarly, wet faeces contaminated initially at 10^2 and 10^4 cfu/g failed to reveal *rR S. typhimurium* after treatment at 2,000 g.h/m^3.

In the wet and "wetter" faeces samples contaminated with 10^6 cfu/g, a drop of at least 3 $\log_{10}$ was observed in sample 3 where contamination was reduced to below 10^2 cfu/g.

In sample 1 however, despite a reduction of 2 $\log_{10}$ in the salmonella count, the MB treatment was not sufficient to provide control (bacterial

colonies at a level of 10^3 cfu/g were recorded after the treatment).

DISCUSSION AND CONCLUSION

The objective of obtaining total disinfection was not achieved. However, the results of this investigation show that MB fumigation it is possible to reduce the salmonella count using a minimum Ct product of 1,500 g.h/m^3 at 18°C, while at a Ct product of 2,000 g.h/m^3, no salmonella were isolated except from the most contaminated faeces.

Table 3: Efficacy of methyl bromide against *S. typhimurium*.

Samples	No.	Level of Contamination (cfu/g)	Control sample (cfu/g)	Ct Products g.h/m^3 1,500	2,000
Wet faeces at 70% m.c.	1	5.5 x 10^6	5.9 x 10^5	5.2 x 10^3	5.2 x 10^3
	2	5.5 x 10^4	4 x 10^3	0	0
Wet faeces at 50% m.c	3	5.5 x 10^6	5.2 x 10^5	+	+
	4	5.5 x 10^4	2.6 x 10^5	0	0
	5	5.5 x 10^2	5 x 10^5	0	0
Dry litter at 15% m.c.	6	5.5 x 10^6	7.4 x 10^3	+	0
	7	5.5 x 10^4	+	+	0
	8	5.5 x 10^2	+	0	0
Feed 1: 12% m.c.	9	5.5 x 10^3	+	+	0
Feed 2: 12% m.c. (25 kg bag)	10	5.5 x 10^3	+	+	0

+ : < 10^2 cfu/g
0 : *S. typhimurium* was not isolated

These results disagree with those of Harry and Burns Brown (1974) who obtained 100% disinfection with a Ct product of 1,600 g.h/m^3 under optimal conditions. However, the results confirm those of Tucker *et al.* (1974), who obtained a considerable reduction in bacterial colonies without total disinfection using a Ct product of 800 g.h/m^3 and 1,600 g.h/m^3 at 20°C.

Complete suppression of salmonella was achieved in samples at a concentration 3 $\log_{10}$ greater than the initial samples using a Ct product of 1,500 g.h/m^3, indicating that actively growing salmonella are more sensitive to MB.

Given that a net reduction of bacterial colonies is obtained using a Ct product of 800 g.h/m^3 (Tucker *et al.*, 1974), it would be interesting to carry

out trials using 2 or 3 consecutive fumigations at a lower Ct product, at different water activities, temperatures, and levels of contamination (Maag and Schmittle 1962).

Another possible application involves the prevention of salmonella infections encountered currently in dried fruits and spices, where ethylene oxide was used in EEC countries until 1990. Now that ethylene oxide has been banned, the only bactericide technique remaining is gamma irradiation. This technique is not always accepted and sometimes has an adverse effect on quality. Possibly, MB could be employed as a substitution for ethylene oxide in this bactericidal role.

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