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THE CONTROL OF BACTERIAL CONTAMINATION IN CARCASS MEAL WITH PROPIONIC ACID

J. J. VAN STADEN, H. N. VAN DER MADE and EILEEN JORDAAN, Veterinary Research Institute, Onderstepoort 0110

ABSTRACT

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The effect of the addition of 2, 3, 5, 7 and 10% respectively of propionic acid on known numbers of bacteria added to or contained in carcass meal was examined. *Escherichia coli* was totally inhibited by 2% propionic acid, while 5% of the acid both inhibited *Salmonella typhimurium* and brought about a 74,7% reduction in the total aerobic bacterial count.

Résumé

LE CONTRÔLE DE LA CONTAMINATION BACTÉRIENNE DANS LA FARINE DE CARCASSE AVEC L'ACIDE PROPIONIQUE

L'effet d'une addition respective de 2, 3, 5, 7, *et* 10% *d'acide propionique sur un nombre connu de bactéries incorporées ou contenues dans la farine de carcasse a été examiné.* Escherichia coli *fut totalement inhibé par* 2% *d'acide propionique, tandis que* 5% *d'acide inhiba* Salmonella typhimurium *et produisit une réduction de* 74,7% *dans le compte bactérien aérobique total.*

INTRODUCTION

The world-wide shortage of animal protein has created an increasing demand for abattoir by-products such as carcass meal for animal feed, but material used for the manufacture of this product is contaminated and often dangerous. Although the sterilization by heat as required by most authorities is effective in obviating contamination, Morehouse & Wedman (1961) and Tittiger (1971) reported the presence of pathogenic bacteria in by-products, and Tittiger concluded that the degree of recontamination was related to the level of hygiene in the plant and the ease with which this level could be maintained. Work done by Van den Heever & Van der Made (1977) revealed that 24,2% of carcass meal specimens yielded *Salmonella*.

Resterilization of contaminated carcass meal by heat is problematical because most by-product processing plants are not designed to handle this type of material, besides which it does not obviate the possibility of subsequent recontamination in the plant. As a possible solution to these problems, the addition of propionic acid as a chemosterilant was investigated.

MATERIALS AND METHODS

General outline

The carcass meal used in this experiment was from a single batch of commercially available material which contained 55% protein and 5% fat and had a pH of 6,5. The material was divided into 205 specimens of 10 g each and 50 specimens of 9 g each, all of which were sterilized and dehydrated by exposure in a thin layer to infra-red radiation* for 45 min. The specimens were stored at room temperature in sterilized moisture proof honey jars of 150 mℓ capacity until required.

Experimental design

The entire experiment was repeated 5 times and was performed in 5 different groups as set out in Table 1.

As a control test for sterility, dehydrated carcass meal specimens chosen at randon were tested for bacterial growth. Saline* at 2, 3, 5, 7 and 10%, and saline plus propionic acid at 2, 3, 5, 7 and 10% each, were added to specimens of carcass meal (Group 1, Table 1), and, after being shaken vigorously for 3 min, the specimens were tested as controls for bacterial sterility.

To determine the effect of propionic acid on pathogenic and potentially pathogenic bacteria in carcass meal, further specimens were treated with saline and saline plus propionic acid as above (see Table 1, Groups 2–4). The bacteria added to these specimens were pure 18 h cultures of *Salmonella typhimurium* (culture No. 2656), *Escherichia coli* type 078: K80 and atoxigenic *Clostridium perfringens*. Known numbers of these bacteria (see assay on Day 0 on Tables 3, 4 & 5) were added to the specimens 30 min after the saline and saline plus propionic acid treatment. These specimens were mixed by vigorous shaking for 3 min, after which colony-forming units (CFU) of bacteria were assayed.

The effect of propionic acid on naturally contaminated commercial carcass meal was determined as set out in Table 1 (Group 5). Naturally contaminated commercial carcass meal specimens (1 g each) with known total aerobic bacterial count (see assay at Day 0 on Table 6) were added to sterilized specimens (9 g each) 30 min after saline and saline plus propionic acid had been added, as above. These specimens were mixed by vigorous shaking for 3 min, after which CFU of bacteria were assayed.

Assay procedures

Bacterial counts. Bacterial counts were obtained by counting CFU by the method of Miles & Misra (1938). The material to be assayed was diluted with physiological saline**, making 5 tenfold dilutions, 0,05 mℓ of which was placed on blood tryptose agar*** plates. These plates were incubated at 37 °C for 19 h and 33 h for aerobic and anaerobic cultures, respectively, after which CFU counts were made. Anaerobic cultures were incubated in a hydrogen atmosphere.

* Infra-red lamp—Sartorius GMBH, Göttingen

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* 0,9 g % NaCl in aqua dest., pH 7

** Sodium chloride solution 0,9 g %

*** Difco, Michigan, U.S.A.

TABLE 1 Experimental design

Group	Composition of specimens	Mass of each specimen (g)	Number of specimens monitored at percentage additions of saline, or saline and propionic acid											Total
			0%	2%		3%		5%		7%		10%		
				saline only	saline +pac	saline only	saline +pac	saline only	saline +pac	saline only	saline +pac	saline only	saline +pac	
1	Sterile control of Scm.....	10	5	5	5	5	5	5	5	5	5	5	5	55
2	Scm+*Salmonella typhimurium*	10	—	5	5	5	5	5	5	5	5	5	5	50
3	Scm+*Escherichia coli*.....	10	—	5	5	5	5	5	5	5	5	5	5	50
4	Scm+*Clostridium perfringens*...	10	—	5	5	5	5	5	5	5	5	5	5	50
5	Scm + contaminated carcass meal.....	9+1	—	5	5	5	5	5	5	5	5	5	5	50

Scm: sterile carcass meal
pac: propionic acid

CFU counts on pure cultures and specimens artificially contaminated with these cultures were done simultaneously to obtain the true numbers of CFU used. For total bacterial counts on naturally contaminated carcass meal specimens, 1 g samples, removed under aseptic conditions, were each suspended in 10 mℓ of saline and shaken vigorously by hand for 1 min, after which further dilutions were made as above for aerobic bacterial counting.

The pH of carcass meal was measured by means of a pH meter* on samples (1 g) which were suspended in 10 mℓ of saline (pH 7) for 30 min.

Statistical methods

For stabilization purposes the data were transformed to logs.

The relationship of time as the independent variable and bacterial count as the dependent variable was examined by means of polynomial regression. The raw data were also examined to determine the developmental tendencies of bacterial numbers over time.

For the calculation and presentation of the inhibition of bacterial numbers by propionic acid, Simpson's method (Granville, Smith & Longley, 1958) was used, employing numerical integration in the calculation of a surface bounded by a graph for the series of independent observations for specimens with and without acid. This calculation for percentage acid effect was done as follows:

$$\% \text{ acid effect} = \frac{NA_{surface} - PA_{surface}}{PA} \times 100$$

where NA = non-propionic acid-treated controls
PA = propionic acid-treated specimens.

Mean values of per cent acid effect for 5 cultures of each bacterium were calculated for each of the different levels of treatment of the specimens with propionic acid. These mean values were plotted against percentage propionic acid, and for this purpose the former were expressed as negative numbers on the graph.

Results

pH measurements

The pH of the carcass meal specimens changed immediately on the addition of propionic acid, and no further change occurred during the 14-day period of observation. The mean pH values of the specimens are presented in Table 2. There was a linear decrease in pH from pH 6,5 of normal carcass meal to pH 5,3 at the 7%, and pH 5 at the 10% propionic acid level.

The results of bacterial counts

The results of the bacterial counts are presented in Tables 3, 4, 5 & 6.

In the control test for sterility (Table 1, Group 1), no bacteria could be cultured from either the sterilized carcass meal, the sterilized carcass meal to which saline had been added or from the sterilized carcass meal to which both saline and propionic acid had been added.

Artificially contaminated carcass meal showed a general tendency for the numbers of bacteria in both acid-treated and non-acid-treated specimens to decrease over the first 24 h, except that the total bacterial count tended to increase initially in some specimens. A typical curve illustrating the tendencies in propionic acid-treated and untreated specimens over the 14-day period is presented in Fig. 1.

* Metrohm, Herisaue E. 488

TABLE 2 Mean pH measurements of carcass meal with saline, and saline plus propionic acid

Addition of saline or saline +propionic acid	pH measurement of carcass meal with:	
mℓ/100 g of carcass meal	Saline only	Saline +pac
2	6,5	6,4
3	6,5	6,2
5	6,5	5,7
7	6,5	5,3
10	6,5	5,0

pac = propionic acid

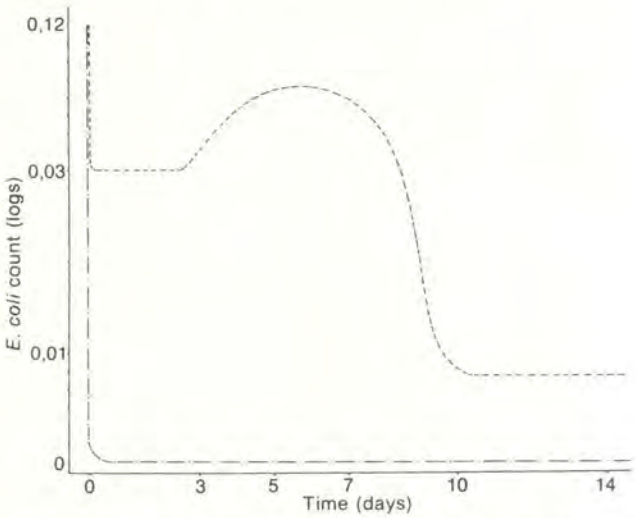

FIG. 1 The bacterial count over 14 days of *E. coli* added to carcass meal, with and without the addition of propionic acid
Without propionic acid ----------,
Addition of 2% propionic acid on Day 0 —·—·—·—·

The total decrease in bacterial numbers which took place during the first 24 h in propionic acid-treated specimens was much higher and much more sudden than in non-acid-treated specimens. Polynomial regression, using time as the independent and bacterial count as the dependent variable, with a few exceptions, resulted in non-significantly fitted curves.

The per cent acid effect against percentage of acid is presented in Fig. 2.

In specimens artificially contaminated with *E. coli*, the mean percentage decrease in bacterial numbers over 14 days at 2% and 3% addition of propionic acid was 99,8% and 99,9%, respectively. Similarly, *S. typhimurium* was reduced by 99,9% by adding 5% of propionic acid to the specimens. Elimination of both these bacteria was achieved at a 7% propionic acid level.

TABLE 3 Mean bacterial counts (CFU) of sterile carcass meal artificially contaminated with *Salmonella typhimurium* (Table 1, Group 2)

Days assayed	Mean numbers of bacteria (CFU)/g assayed at percentage additions of saline, or saline and propionic acid									
	2%		3%		5%		7%		10%	
	saline only	saline +pac	saline only	saline +pac	saline only	saline +pac	saline only	saline +pac	saline only	saline +pac
0	8×10^6	8×10^6	8×10^6	8×10^6	8×10^6	8×10^6	8×10^6	8×10^6	8×10^6	8×10^6
1	2×10^7	2×10^6	4×10^7	6×10^5	3×10^6	4×10^2	2×10^7	0	1×10^7	0
3	1×10^6	3×10^6	2×10^7	1×10^6	9×10^6	4×10^2	1×10^7	0	3×10^7	0
5	9×10^6	7×10^5	3×10^7	1×10^5	2×10^7	4×10	7×10^6	0	8×10^5	0
7	1×10^7	4×10^6	2×10^7	1×10^5	8×10^6	0	1×10^7	0	8×10^5	0
10	8×10^6	4×10^5	2×10^7	1×10^5	2×10^6	4×10	4×10^6	0	1×10^7	0
12	6×10^6	1×10^5	2×10^7	3×10^4	1×10^6	0	9×10^5	0	7×10^5	0
14	1×10^7	3×10^6	1×10^7	7×10^4	3×10^6	0	6×10^6	0	2×10^5	0

pac=propionic acid

TABLE 4 Mean bacterial counts (CFU) of sterile carcass meal artificially contaminated with *Escherichia coli* (Table 1, Group 3)

Days assayed	Mean numbers of bacteria (CFU)/g assayed at percentage additions of saline, or saline and propionic acid									
	2%		3%		5%		7%		10%	
	saline only	saline +pac	saline only	saline +pac	saline only	saline +pac	saline only	saline +pac	saline only	saline +pac
0.....	2×10^6	2×10^6	2×10^6	2×10^6	2×10^6	2×10^6	2×10^6	2×10^6	2×10^6	2×10^6
1.....	2×10^6	6×10^3	2×10^6	4×10^2	2×10^6	0	1×10^6	0	8×10^5	0
3.....	3×10^6	4×10^3	3×10^6	2×10^2	2×10^6	0	8×10^5	0	7×10^5	0
5.....	1×10^6	2×10^3	1×10^6	8×10	2×10^6	0	4×10^5	0	4×10^5	0
7.....	2×10^6	8×10^2	2×10^6	4×10	8×10^5	0	8×10^5	0	3×10^5	0
10.....	8×10^5	5×10^2	1×10^6	0	4×10^5	0	3×10^5	0	8×10^4	0
12.....	7×10^5	2×10^2	8×10^5	4×10	6×10^5	0	8×10^5	0	3×10^5	0
14.....	6×10^5	4×10	8×10^5	4×10	6×10^5	0	3×10^5	0	2×10^5	0

pac=propionic acid

TABLE 5 Mean bacterial counts (CFU) of sterile carcass meal artificially contaminated with *Clostridium perfringens* (Table 1, Group 4)

Days assayed	Mean numbers of bacteria (CFU)/g assayed at percentage additions of saline, or saline and propionic acid									
	2%		3%		5%		7%		10%	
	saline only	saline +pac	saline only	saline +pac	saline only	saline +pac	saline only	saline +pac	saline only	saline +pac
0.....	4×10^4	4×10^4	4×10^4	4×10^4	4×10^4	4×10^4	4×10^4	4×10^4	4×10^4	4×10^4
1.....	3×10^4	1×10^3	1×10^4	3×10^2	2×10^4	5×10^2	7×10^3	0	5×10^3	2×10^2
3.....	3×10^3	6×10^2	3×10^3	2×10^2	2×10^4	2×10^2	1×10^3	4×10	1×10^3	4×10
5.....	8×10^2	9×10^2	9×10^2	6×10^2	2×10^3	2×10^2	8×10^2	1×10^2	6×10^2	4×10
7.....	2×10^3	5×10^2	3×10^3	5×10^2	2×10^3	4×10^2	9×10^2	4×10^2	2×10^3	4×10^2
10.....	7×10^2	2×10^2	2×10^3	2×10^2	2×10^3	2×10^2	8×10^2	1×10^2	6×10^2	1×10^2
12.....	6×10^2	9×10^2	1×10^3	6×10^2	1×10^3	2×10^2	6×10^2	1×10^2	6×10^2	2×10^2
14.....	2×10^3	2×10^2	2×10^3	5×10^2	1×10^3	1×10^2	8×10^2	1×10^2	7×10^2	2×10^2

pac=propionic acid

TABLE 6 Mean total aerobic bacterial counts (CFU) of sterile carcass meal (9 g) mixed with contaminated carcass meal (1 g) (Table 1, Group 5)

Days assayed	Mean numbers of bacteria (CFU)/g assayed at percentage additions of saline, or saline and propionic acid									
	2%		3%		5%		7%		10%	
	saline only	saline +pac	saline only	saline +pac	saline only	saline +pac	saline only	saline +pac	saline only	saline +pac
0.....	4×10^5	4×10^5	1×10^6	1×10^6	5×10^4	5×10^5	5×10^5	5×10^5	5×10^5	5×10^5
1.....	1×10^5	6×10^4	7×10^5	7×10^5	2×10^5	4×10^5	1×10^5	3×10^3	2×10^5	2×10^2
3.....	2×10^5	7×10^4	1×10^5	3×10^5	1×10^5	8×10^4	7×10^4	3×10^3	1×10^5	5×10^2
5.....	1×10^5	2×10^5	1×10^5	8×10^4	5×10^4	1×10^4	1×10^5	3×10^3	1×10^5	1×10^3
7.....	8×10^5	2×10^5	2×10^5	1×10^5	1×10^5	2×10^3	1×10^5	1×10^3	1×10^5	3×10^2
10.....	1×10^5	4×10^4	1×10^5	2×10^4	1×10^5	1×10^4	8×10^4	6×10^2	9×10^4	3×10^2
12.....	1×10^5	7×10^4	2×10^5	2×10^4	1×10^5	8×10^4	1×10^5	1×10^3	2×10^5	6×10^2
14.....	8×10^4	8×10^4	2×10^5	3×10^4	1×10^5	9×10^3	1×10^5	7×10^2	9×10^4	4×10^2

pac=propionic acid

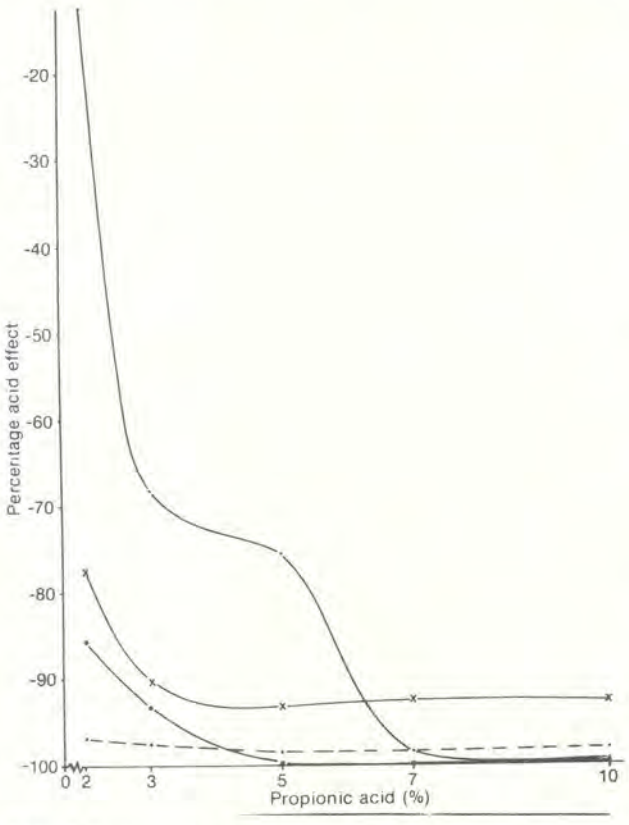

FIG. 2 Presentation of percentage of propionic acid in carcass meal against percentage acid effect on bacterial numbers
Total aerobic bacterial count ·————·
E. coli ·- - - - -·, Clostridium x————x, Salmonella o————o

The total bacterial count was reduced by 74,77% and 99,8% by the addition of 5% and 10% of propionic acid, respectively. The numbers of viable *Clostridia* were reduced by 94,05% over the 14-day period at the 3% propionic acid level, but very little additional reduction was achieved by increasing this level to 10%

Discussion

The results obtained in this experiment indicate that 7% of propionic acid added to carcass meal is very useful as a decontaminant. This level of propionic acid is harmless to non-ruminants, and acts as a direct source of energy to ruminants (Armstrong, 1965; Cole, Beal & Luscombe, 1968).

The application of propionic acid in the processing plant should occur at a point after heat sterilization and centrifugation, but before milling, to ensure thorough mixing with the product. The pH of carcass meal should be monitored as a control for the amount of propionic acid added. An investigation should be undertaken into the application of propionic acid as a decontaminant to other animal feed products such as bone meal and blood meal.

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