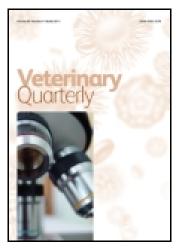
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Lactic acid as a decontaminant in slaughter and processing procedures

J. M. A. Snijders, J. G. van Logtestijn, D. A. A. Mossel, and F. J. M. Smulders¹

SUMMARY An attempt was made to interrelate the data obtained in experiments conducted by our Department along beef, veal and pig slaughter lines, using lactic acid (LA) for the decontamination of carcasses, cold and hot boned primal cuts, slaughter byproducts, and butchers' knives.

First and foremost it was observed, that provided Good Manufacturing Practices are strictly followed, the microbial load of carcass surfaces will be substantially reduced. LA-decontamination may result in an additional reduction. Since in the early post-mortem period bacteria are not yet attached to the meat surface, LA-decontamination should preferably be applied to the hot carcass. It was demonstrated that, dependent on mode and duration of application, LA sprays not exceeding 1% v/v (beef), 1.25% v/v(veal) and 1.5% v/v (pork) resulted in acceptable carcass colour scores. Blood spots, which are particularly prone to discolouration by lactic acid application, should be removed at an early postmortem stage e.g. by strong showering. The difference in surface pH between LA-treated and control carcasses disappeared within 72 hours post-mortem. Veal longissimus chops treated with LA solutions up to 2% v/v were not identified by a consumer taste panel as significantly different from controls. The 'immediate' bactericidal effect of LA-decontamination for beef, veal and pig carcasses, as well as

The 'immediate' bactericidal effect of LA-decontamination for becy, real and pig carcusted, as well as for pig liver and veal brain, amounted to approximately 1.5 log cycles for the aerobic colony counts, strongly dependent on substrate and conditions of decontamination. In addition, a 'delayed' bacteriostatic effect was observed during storage, which is probably the result of a prolonged lag phase of acid-injured micro-organisms surviving lactic acid decontamination.

Ecological surveys revealed that LA resulted in a shift towards a Gram positive bacterial association acting as an antagonist of enteropathogenic Gram negative bacteria. Electrostatic application of LA solutions may contribute to limiting the amount of LA needed for effective decontamination.

Adding 2.7% v/v LA to the spray water of a specially designed disinfection unit for butchers' knives effected a reduction in aerobic colony counts at 45° C which exceeded that achieved by conventional sanitizers at 82° C.

INTRODUCTION

Good manufacturing practices (GMP) during slaughter include all measures necessary to produce meat with the lowest possible microbial contamination. This is attainable only if the whole process is strictly controlled (16).

Microbiological examination of carcass surfaces should be used to identify critical hazard points in slaughter lines (3). Data thus obtained may be used in steering the processs in order to attain a satisfactory final product.

Usually, pig skin is heavily contaminated with bacteria. During scalding and dehairing and particularly during singeing a substantial reduction of the bacterial load is achieved (12). In the blackscraper and polishing machinery, however, pig carcasses are often severely recontaminated (3, 12). If cleaning and disinfection are inadequate, considerable quantities of dirt (hair, parts of the epidermis, etc.) remain in the machinery. Consequently the blackscraping and polishing machinery acts as a continuous source of bacterial contamination.

In cattle and veal slaughter lines the skinning process entails severe hygienic problems. Mechanical skinning is therefore preferable. Evisceration is another critical point. Carcasses should be opened carefully to prevent intestinal perforation. The rectum should be excised and tied off. Furthermore, an integral part of GMP is the training, instruction, and motivation of personal working on slaughter lines (4).

¹ Faculty of Veterinary Medicine, Department of the Science of Food of Animal Origin, Faculty of Veterinary Medicine, State University of Utrecht, P.O. Box 80 175, 3508 TD Utrecht, The Netherlands. Decontamination is another possibility for reducing the contamination of carcasses, cuts, and byproducts. However, it has often been questioned whether decontamination procedures are really required when advanced hygiene measures and adequate refrigation are applied along slaughtering and processing lines. Whenever and wherever, in spite of hygienic practices, meat surfaces or cuts do become (cross) contaminated, such a terminal decontamination may be useful (9).

Lactic acid (LA) is an acceptable decontaminant, because i) it is a natural product; ii) it is physiological and not toxic; iii) it is often used in the meat industry, and is also produced fortuitously in meat products as a result of fermentation (2).

This paper is an attempt to interrelate the data obtained by our Department in several experiments on lactic acid decontamination.

MATERIALS AND METHODS

Experiments were conducted on pig, veal, and cattle slaughter lines.

Carcasses were sprayed with LA solutions at approximately 45 minutes post-mortem, and subsequently chilled in conventional chill rooms ($3 \pm 1^{\circ}$ C). In one experiment hot deboned pig bellies were sprayed with 5% v/v lactic acid, 3 hours post mortem, and subsequently stored under refrigeration at 7° C for 8 days. Porcine livers were dipped for 5 minutes in a 0.20% v/v lactic acid solution, allowed to drain for 30 s, and subsequently vacuum packaged and stored at $3 \pm 1^{\circ}$ C. Veal brains were sprayed with 1.25 LA after manual extirpation. In a special cleaning and disinfection unit (a perspex tube with a diameter of 6 cm and two flat spray nozzles, Figure 1) tests with and without LA were carried out on stainless steel plates. LA solutions were prepared by diluting a 90% L-lactic acid stock solution (Chemic Combinatie Amsterdam).

The surface pH of carcasses was assessed with a pH meter and combined electrodes (Russel pH Ltd, Auchtermuchty, Fife, Scotland). The colour score of carcasses after LA treatment was assessed visually by two trained graders. Veal longissimus chops were evaluated sensorically by a 15 member consumer panel.

Sampling for bacteriological examination was carried out using an excision technique (12, 13, 15). Tissue discs were sampled by means of sterile cork borers, scalpels, and tweezers, whereupon they were collected in plastic bags.

After peptone-saline solution had been added, samples were macerated in a Stomacher for 2 minutes. Colony counts were expressed in colony forming units (c.f.u.) per cm² (except those from veal brains which were expressed per gram) and then converted to logarithms base 10. The following criteria for meat samples were determined: Mesophilic aerobic colony counts (ACC) in plate count agar or Tryptone Glucose Beef Extract Agar (poured plate method, incubation at 30° C for 3 days); Enterobacteriaceae colony count (ECC) in violet red bile glucose agar (poured Plate method with overlayer, incubation at 37°C for 1 day).

As a substitute for knifeblades, stainless steel plates (9 x 4.5 cm) were used. They were overlayered with buffered Tryptone Soya Broth Agar (Direct Surface Agar Plate method (1)). For this examination colony counts were expressed in c.f.u. per knife. Differences between c.f.u. counts were assessed using a Student t-test and a Wilcoxon test.

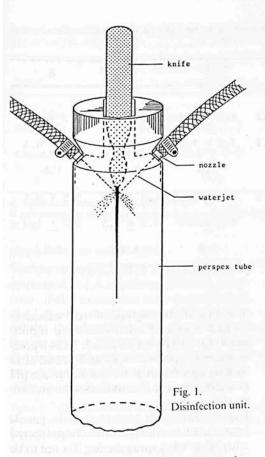
RESULTS AND DISCUSSION

Bacteriological data

The importance of cleaning and disinfection as part of GMP is clearly shown in Table 1. Aerobic colony counts of 4.2 log N per cm² were found on pork skins after they had passed polishing machinery which had not been cleaned appropriately (12).

When cleaning was carried out more systematically but without daily disinfection, the contamination of the carcasses was reduced to 3.3 log N per cm². Mechanizing the cleaning procedure by using rotating spraying devices combined with a daily disinfection programme resulted in a further reduction of contamination. When the carcasses were sprayed after having passed the polishing machinery with a 1% v/v lactic acid solution (2 L of solution for 25 sec per carcass), an additional reduction of 0.5 log N per cm² was obtained. The effect of LA decontamination depends, among other things, on concentration, application time, temperature, the attachment of micro-organisms to surfaces, and the application method e.g. spraying or immersing (6, 8). A 5% LA spray results in a high reduction viz. $\triangle = 0.9$ -3.4 (Table 2), but causes an unacceptable discolouration of the treated cuts.

The data in Table 2 show also very clearly the delayed bacteriological effects of LA decontamination on hot deboned pig bellies under experimentally inadequate storage conditions (7° C). Especially Enterobacteriaceae were strongly inhibited in their development. In further studies (7) it was found that LA decontamination effects a shift on the microflora towards the Gram positive population and provides a greater protection against the possible enteropathogenic Gram negative association.



As shown by the data in Table 3, spraying of hot carcasses at 45 minutes p.m. resulted in a greater effect than spraying of chilled carcasses. This is probably due to the fact that on hot carcasses the bacteria are still in the waterfilm and have not yet become attached to the surface (8).

The breast of a cattle carcass is signifi-

cantly more contaminated than the shoulder (Table 3). Therefore lactic acid decontamination is most effective at the former site (13). After 3 days chilling some delayed effect of lactic acid decontamination is still noticeable, although not as clearly as in the experiments on pigs, shown in Table 2.

This delayed effect, which is probably the result of a prolonged lag phase of acid-injured micro-organisms surviving LA decontamination, is also observed in veal carcasses during prolonged storage time (Table 4) (10).

Organoleptic acceptability and pH

Experiments have shown that the optimal concentration for LA spraying which does not cause permanent discolouration of the meat amounts to 1% v/v for beef and 1.25% v/v for veal. However, immediately after LA spraying a slight discolouration occurs, which disappears after 1 day of chilling. The acceptable LA concentration is slightly higher for veal than for beef, because veal is paler. Nevertheless, LA concentrations exceeding 1.25% v/v will produce undesirable discolouration of the subcutaneous fat cover on the carcass. On the other hand, after this fat has been trimmed off primal cuts may be treated with concentrations as high as 2% v/v without causing discolouration (10).

High concentrations of LA caused blood to coagulate, leaving rusty brown spots which are commercially unacceptable. To prevent this brown discolouration, carcasses must be rinsed before treatment in order to remove the blood spots.

Table 1. The effect of cleaning and disinfection of the polishing machinery with or without an additional 1% v/v lactic acid spray on the carcasses (applied 45 min p.m.) on the aerobic colony counts (ACC) of pig carcasses.

Treatment	ACC in log N/cm ²
1. Irregular cleaning	4.2 <u>+</u> 0.5 ^{* a}
2. Systematic foam cleaning	3.3 <u>+</u> 0.3 ^b
3. Mechanisation of foam cleaning and disinfection	2.8 <u>+</u> 0.3 ^c
4. as 3, and subsequently LA spray	2.3 <u>+</u> 0.4 ^d

Each mean value comprises 20 observations. From each carcass samples were pooled from the rind on the cheek, the breast and the back. The values with different superscripts differ significantly (p < .01).</p>

Days of stora	age O	2	3	6	8
ACC					
controls	4.7 + 0.4	7.4 ± 0.2	8.0 + 0.1	8.7 <u>+</u> 0.3	9.2 + 0.1
treated	3.8 ± 0.4	4.0 + 0.7	5.2 + 1.3	6.4 <u>+</u> 0.7	7.6 <u>+</u> 0.5
Δ	0.9	3.4	2.8	2.3	1.6
ECC					100 000 10 000 0
controls	2.7 ± 0.5	3.1 ± 1.5	4.6 + 0.6	6.0 <u>+</u> 0.5	5.5 <u>+</u> 0.4
treated	< 1.3	< 1.3	1.7 ± 0.7	2.7 + 1.5	3.3 <u>+</u> 1.3
Δ	≥ 1.4	<u>≥</u> 1.8	2.9	3.3	2.2

Table 2. The effect of 5% v/v lactic acid (applied 3 h p.m.) on aerobic (ACC) and Enterobacteriaceae colony counts (ECC) (log N/cm²) of hot deboned pig bellies stored at 7° C.

 Δ significant reduction (p < .01) n = 8

For pork the optimal LA concentration is also 1% v/v. But if little liquid is used, for instance as a result of applying electrostatic spraying, concentrations of up to 1.5% v/vcan be used (5). The difference in LA concentrations resulting in minimal discolouration may be attributed to dilution of LA in the waterfilm covering pig carcasses. A 2.4% v/v LA spray on pork carcasses produces clear discolourations, especially in the thorax, cavities, and locations in which lactic acid solutions are collected. The pH of the surface of veal carcasses treated with 1.25% LA decreased significantly (p < .01) from 7.0 to 3.7. At 24 h postmortem the pH was back at 5.6, and after 72 h no significant difference in surface pH could be observed between treated and untreated carcasses.

Experiments with consumer taste panels have shown veal longissimus chops treated with 2% v/v LA spray during 30 s not to be significantly different from the controls, whereas a treatment with 4% v/v LA could be identified (18).

Table 3. The effect of a 1% v/v lactic acid spray (applied 45 min p.m.) on the bacteriological condition of cattle carcasses.

Days	Aerobic col O	ony count 3	Enterobacte 0	riaceae colony count 3
Breast controls	4.7 <u>+</u> 0.4	5.3 <u>+</u> 0.9	2.0 <u>+</u> 0.6	75% [*] 2.1 <u>+</u> 0.8
treated	2.9 + 0.8	2.9 + 0.7	~ 1.3	< 1.3
Δ	1.8	2.4	<u>≥</u> 0.7	<u>≥</u> 0.7
shoulder controls	3.1 <u>+</u> 0.7	3.9 <u>+</u> 1.4	13% 1.9	13% 2.2
treated	2.5 ± 0.7	2.6 + 0.8	< 1.3	. < 1.3
Δ	0.6	1.3		

 Δ significant reduction (p < .05) n = 8

★ percentage of plates appropriate for enumeration from which means have been calculated (% : log N/cm² \ge 1.3)

Table 4. The effect of a 1.25% v/v lactic acid spray (applied 45 min p.m.) on the bacteriological condition (log N/cm²) of veal carcasses.

	Aerobic colony count		Enterobacteriaceae colony count	
Days	0	14	0	14
shoulder controls	3.2 <u>+</u> 0.5	4.6 + 1.2	242 [*] 1.9 <u>+</u> 0.4	29% 2.7 <u>+</u> 0.2
treated	2.5 <u>+</u> 0.5	3.3 <u>+</u> 0.8	6% 1.3	6% 2.2
Δ	0.7	1.3	≥ 0.6	≥ 0.5

 \triangle significant reduction (p < .01) n = 17

* percentage of plates appropriate for enumeration from which means have been calculated (% : log N/cm² \geq 1.3)

Application on edible offals

Besides spraying, cuts or organs can also be immersed in LA solutions (Table 5). However, if the immersion time is prolonged, the LA concentration must be reduced to inhibit discolouration (17). Spraying veal brains with 1.25% v/v LA gave a small immediate effect (reduction of 0.3 log N per g), but should be discouraged because after one week of storage brains showed an unacceptable discoloration, whereas the bacterial load was not significantly different from that of controls (11).

Effect on knives

According to EEC regulations the temperature of water used for cleaning and disinfection of tools during slaughtering has to be at least 82° C (180° F).

Table 5. Combined effect of decontamination by immersion in 0.20% v/v lactic acid for 5 min followed by vacuum packing on the bacteriological condition at the surface of porcine liver (log N/cm²).

	Aerobic colony count		Enterobacteriaceae colony count		
Days	1	5	and see with the set	5	
Controls	4.4 + 0.4	5.2 + 0.5	2.1 + 0.6	2.2 ± 0.6	
treated	2.2 <u>+</u> 0.3	2.4 + 0.6	17% [*] 1.7 <u>+</u> 0.1	17%1.5 + 0.1	
Δ	2.2	2.8	<u>≥</u> 0.4	<u>≥</u> 0.7	

 Δ significant reduction (p < .001) n = 12

★ percentage of plates appropriate for enumeration from which means have been calculated (% : log N/cm² \geq 1.3)

Table 6. The effect on the aerobic colony count of cleaning and disinfection of knives in a desinfection unit with or without 2.7% v/v lactic acid and a pressure of 15 atm at different temperatures and spraying times.

time S	temperature C	reduction ∆ log N	reduction with LA ∆ log N
5	20	0.2 ^{* a}	2.6 ^c
5	20	0.4 ^a	3.2 ^d
5	45	0.7 ^b	3.1 ^d

* Each mean value comprises 20 observations.

The values with different superscripts differ significantly (p < .01). The mean level of contamination on the knives which were used as controls was $3.3 \pm 0.2 \log N$.

Immersing a knife for 15 sec in water of 82°C effects a reduction of 2.2 log N, which is significantly lower than the reduction obtained in the disinfection unit with LA (Table 6). Disinfection of knives at 82° C is far from optimal if it is not preceded by mechanical cleaning. The latter is possible if one uses a disinfection unit in which water is sprayed onto the knife surface through 2 nozzles (14). By adding LA to water a lower temperature may be used, and disinfection by spraying resulted in a better effect than was obtained in standing water.

CONCLUSION

The use of LA as a terminal decontaminant linked to perfect slaughter line hygiene could bring important advantages. LA produces both an immediate (batericidal) and a delayed (bacteriostatic) effect, which results in an extended shelf life of meat. The level of contamination with enteropathogenic micro-organisms may be reduced through an increased suppression of the Gram negative bacteria. However, the use of lactic acid as a decontaminant in the meat industry must never result in neglect of hygiene in slaughtering and processing lines.

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