Reprinted from
Journal of Food Protection, Vol. 51, No. 11, Pages 869-873 (November 1988)
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Reduction of Bacteria Attached to Meat Surfaces by Washing with Selected Compounds

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(Received for publication February 24, 1988)

ABSTRACT

Beef muscle and fat tissue were inoculated with either *Listeria monocytogenes, Salmonella typhimurium, Staphylococcus aureus,* or *Serratia marcescens* and washed with various compounds. There were no significant (P>0.05) differences in detachment and/or destruction of bacteria from lean tissue with phosphate buffer, ethanol, and NaCl, with all of the reductions being <1 log cycle. NaOH and KOH were effective in removing bacteria from both fat and lean tissue, reducing the populations of *S. typhimurium* and *S. marcescens* on the tissue by as much as 4 log cycles. For all of the bacteria tested, there were significantly more (P<0.05) bacteria removed or destroyed from fat tissue than from lean tissue. There was no significant difference (P>0.05) between NaOH and KOH. Concentration (1%, 5%, and 10%) of the wash solution had a significant (P<0.05) effect on all four of the bacteria. D values were determined for the bacteria in the three concentrations of NaOH. The values ranged from a high of 41 sec for *S. aureus* in 1% to <1.2 sec for *L. monocytogenes* in 10% NaOH.

Animal carcasses are known to be contaminated with a variety of pathogenic bacteria, including *Salmonella (9), Campylobacter (5), Escherichia coli (10),* and *Listeria (7).* A variety of methods or treatments have been proposed to control these pathogens. Proper slaughtering and dressing procedures, combined with good sanitation, are effective in preventing initial contamination of the carcass *(4,6).* Other researchers have investigated the use of washes or sprays to remove or destroy surface contamination on the animal carcasses *(2).* Most of this research has focused on the use of high pressure water rinsing or the use of chemical sanitizers.

High pressure washing with only water (no sanitizer) has been found to reduce the total aerobic and *Enterobacteriaceae* counts on beef by approximately 1 *(3)* and 1.5 log cycles *(8),* respectively. Some of the sanitizing compounds which have been evaluated include chlorine and organic acids, such as acetic and lactic. Stevenson et al. *(21)* reported that carcasses washed with 200 mg/L sodium hypochlorite spray had lowered total aerobic counts from the controls, but that these differences were not significant. Several researchers have demonstrated the bactericidal effects of organic acids on the microflora of meat *(17,19,23),* although the reductions in total aerobic counts have generally not been significant *(1,15).* The use of alkaline solutions has been explored by Humphrey et al. *(13),* who reported that poultry scald water adjusted to pH 9 with NaOH significantly reduced the total aerobic counts on poultry carcasses.

Most of the previous research has focused on intact carcasses. The differences, if any, between beef fat and lean tissue have not been adequately addressed. In addition, other compounds have not been evaluated for their effectiveness in meat systems. The objective of this study was to determine the effect of ethanol, NaCl, NaOH and KOH on bacteria on fat or lean tissue surfaces.

MATERIALS AND METHODS

Bacterial cultures

Cultures of *Salmonella typhimurium* (ATCC 14028), *Serratia marcescens* (ATCC 8100), *Staphylococcus aureus* (ATCC 25923), and *Listeria monocytogenes* (strain Scott A; Bacterial Physiology Branch, Division of Microbiology, FDA, Cincinnati, OH) were grown and maintained in tryptic soy broth (TSB, Difco at 37°C). Cultures were inoculated approximately 18 h prior to use, and incubated quiescently at 37°C with the exception of *L. monocytogenes,* which was incubated at ambient temperature (ca. 23°C).

Washing experiments

Solutions of NaCl, KOH, and NaOH (1%, 5%, and 10%; Fisher Scientific) were prepared on a wt/vol basis with distilled water and sterilized by autoclaving. The ethanol solutions (5%, 10%, and 20%) were prepared on a vol/vol basis with distilled water and filter sterilized through a 0.45 μm nucleopore filter. All solutions were prepared in 20-ml volumes in 25mm x 150mm glass tubes.

[1]*Mention of trade names, proprietary products or specific equipment does not constitute a guarantee or warranty of the product by the USDA and does not imply its approval to the exclusion of other products that may also be suitable.*

Preparation of meat tissue

Fresh lean muscle and fat tissue (boneless beef trim) were obtained from the abattoir at the U.S. Meat Animal Research Center. Tissues were cut into 0.5-cm thick slices and frozen in sterile bags (Nasco, Fort Atkinson, WI) until use (maximum of 4 weeks). Immediately prior to use 1.0 x 1.0 x 0.5 cm cubes were cut from the tissue slices with a sterile scalpel. These slices typically contained <100 colony forming units (CFU).

The bacteria were harvested by centrifugation (3000G x 10 min. 4°C) and the pellets resuspended in 10 ml Butterfield's phosphate buffer *(11)*. Two ml of the resuspended bacteria was added to 20 ml attachment medium *(16)*, which consisted of 8.7g NaCl, 1.66g Na_2HPO_4, 0.33g NaH_2PO_4, and 0.37g EDTA per liter of distilled water to yield an approximate concentration of 10^7 CFU/ml. The tissue samples were transferred one at a time to the inoculated attachment medium and allowed to stand at ambient temperature for 10 min. After 10 min, the samples were transferred to tubes of the appropriate washing solution and vortexed on a Vortex Genie 2 (Scientific Industries, Bohemia, NY) set at 75% of the maximum speed setting for 10 sec.

Enumeration of bacteria

The washed samples were immediately stomached for 2 min with sterile Butterfield's phosphate buffer in a Stomacher 400 (Tekmar Co., Cincinnati OH). The samples were serially diluted in phosphate buffer and plated on tryptic soy agar (TSA, Difco) using the pour plate technique *(20)*. The plates were incubated at 37°C for 24 h, with the exception of the *L. monocytogenes* plates, which were incubated at 32°C.

Determination of D values

Pure cultures of the bacteria were harvested as outlined above, and 1-ml portions were transferred to 20 ml of the appropriate concentration of NaOH or KOH and vortexed for 5 sec at ambient temperature. At selected time intervals, 1 ml of the mixture was transferred to 99 ml Butterfield's phosphate buffer containing sufficient 1 N HCl (ca. 1.4 ml for 10% solutions) to neutralize the NaOH. Time intervals for transfer (in seconds) were determined by trial and error. The neutralized solution was serially diluted and enumerated using plate count agar (PCA, Difco) and the pour plate technique. Death rate curves were constructed by linear regression, plotting the average log CFU vs. time. When possible, D values were averaged over a three log cycle reduction.

Statistical analysis

Statistical analysis was performed using SAS *(18)*. Unless other wise noted, significance is expressed at the 5% level. Each experiment was performed in duplicate at a minimum, with most being performed in triplicate.

RESULTS

Effects of NaCl, phosphate buffer, and ethanol

The results of the washing experiments with NaCl, phosphate buffer and ethanol on lean tissue are presented in Table 1. The average inoculum level for *L. monocytogenes* and *S. typhimurium* was log 6.95 and 7.12 CFU/sample, respectively. There was no significant difference between the responses of either bacterium to the treatments, irrespective of treatment concentration. The average reduction in the population of *S. typhimurium* was 0.69, 0.66, and 0.61 log cycles for NaCl, phospate buffer, and ethanol, respectively. The average reduction in the population of *L. monocytogenes* was 0.72, 0.61 and 0.54 log cycles for NaCl, phosphate buffer, and ethanol, respectively.

TABLE 1. *Effect of NaCl, phosphate buffer, and ethanol on bacteria attached to lean meat tissue.*

Compound	Concentration	Net Reduction[a] *Salmonella*	*Listeria*
NaCl	1%	0.85	0.79
	5%	0.64	0.84
	10%	0.57	0.52
Ethanol	5%	0.66	0.49
	10%	0.55	0.61
	20%	0.61	0.52
Buffer	NA[b]	0.66	0.61

[a]Log_{10} (control mean) - log_{10} (treatment mean).
[b]Not applicable.

Effects of NaOH and KOH

D values: The average D values for the different bacteria exposed to NaOH are given in Table 2. Similar results were obtained with KOH solutions (data not shown). When no viable cells could be recovered after 10 sec (in excess of a 7 log cycle reduction) an estimated value was calculated assuming a linear decline with time. The theoretical reductions in population for a 10 sec exposure are shown for comparison with the washing treatments for lean and fat tissue shown in Figures 1-4. For every bacterium tested, the 5% and 10% solutions produced virtually instant destruction. *L. monocytogenes* and *S. aureus* were significantly more resistant to the 1% concentration than either *S. typhimurium* or *S. marcescens*.

Salmonella typhimurium: Experiments were carried out using high (ca. 10^7 CFU) and low (ca. 10^4 CFU) inoculum levels on both fat and lean tissue. These results are shown in Figure 1. There was no significant difference between the effects of KOH and NaOH for either of the tissue types. However, there was a significant difference in the effects between fat and lean tissue. With the high inoculum, there was a 2 to 3 log reduction in the bacterial count on fat tissue with the higher concentrations of washing solutions, while the reduction on the lean tissue was in the range of 1 to 2 logs. There were significant differences between the three concentrations at the higher inoculum level.

The results for the lower inoculum level were similar to those of the high level. There was significant difference between 1% and 5% concentrations, although there was no difference between 5% and 10%. Reductions in bacterial numbers ranged from 1 to 2 log cycles with the lean tissue to 2 to 3 log cycles with the fat tissue.

Listeria monocytogenes: The results of the *L. monocytogenes* experiments are shown in Figure 2. These experiments were also carried out with both high and low inoculum levels, similar to those for *S. typhimurium*. The results were similar to those of the *S. typhimurium* experiments. There were no significant differences between KOH and NaOH at either inoculum level, although there was a significant difference between fat and lean tissues. At the high

TABLE 2. *Effect of NaOH concentration on the D values of selected bacteria.*

Concentration of NaOH	D Value[a] (sec)			
	Listeria monocytogenes	*Salmonella typhimurium*	*Staphylococcus aureus*	*Serratia marcescens*
1%	8.13	<1.34	41.51	<1.35
5%[b]	<1.28[c]	<1.34	<1.35	<1.35

[a]Sec. required to reduce population by 1 log cycle.
[b]Results from 10% concentration are identical to 5%.
[c]No viable cells recovered after 10 sec; value estimated assuming a linear decline with time.

Concentration of NaOH	Calculated Reduction[d] for washing treatments			
	Listeria monocytogenes	*Salmonella typhimurium*	*Staphylococcus aureus*	*Serratia marcescens*
1%	1.23	>7.00	0.24	>7.00
5%[e]	>7.00	>7.00	>7.00	>7.00
10%	>7.00	>7.00	>7.00	>7.00

[d]Calculated reduction based on D value and 10 sec exposure, expressed as $\log_{10}$ CFU.
[e]Results from 10% concentration are identical to 5%.

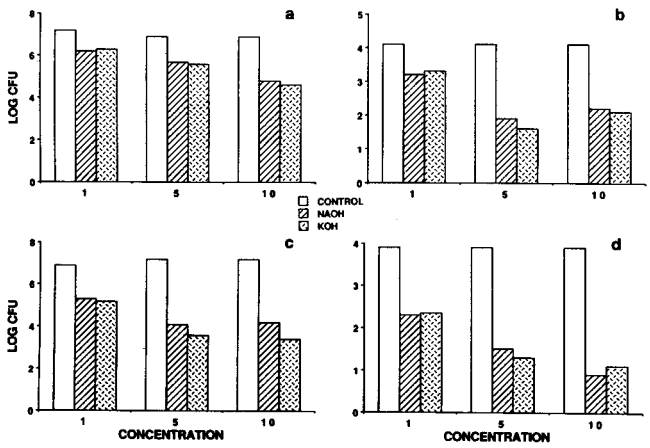

Figure 1. *Effect of NaOH and KOH on* S. typhimurium *attached to lean and fat tissues (log CFU/2 cm²). Concentration expressed as percent. (a) lean tissue (b) lean tissue, low inoculum (c) fat tissue (d) fat tissue, low inoculum.*

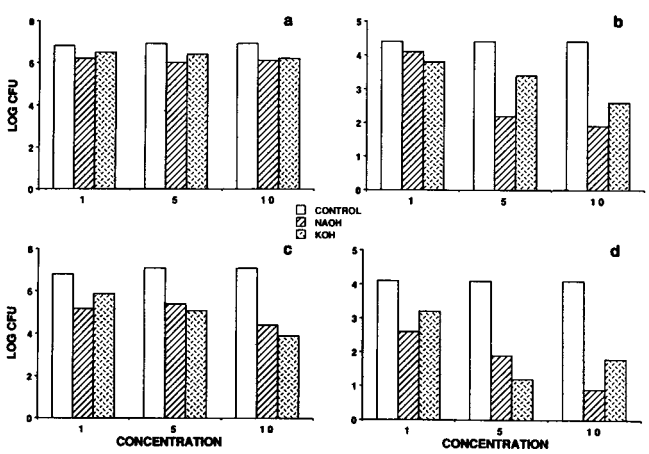

Figure 2. *Effect of NaOH and KOH on* L. monocytogenes *attached to lean and fat tissues (log CFU/2 cm²). Concentration expressed as percent. (a) lean tissue (b) lean tissue, low inoculum (c) fat tissue (d) fat tissue, low inoculum.*

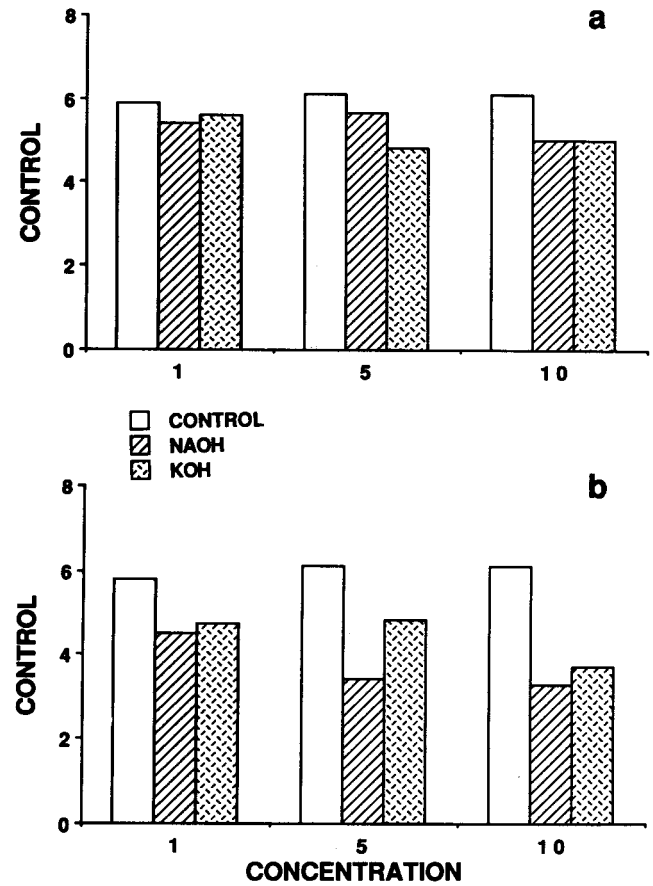

Figure 3. *Effect of NaOH and KOH on* S. aureus *attached to lean and fat tissues (log CFU/2 cm²). Concentration expressed as percent. (a) lean tissue (b) fat tissue.*

inoculum level, there was a significant difference between 1%, 5% and 10% concentrations with the fat tissue. There was no significant difference between the concentrations with lean tissue. The reductions in bacterial counts followed the same pattern as *S. typhimurium*, although the reductions were not as large. Typically, there was less than a 1 log reduction on the lean tissue and approximately a 2 log reduction on the fat tissue with the high inoculum.

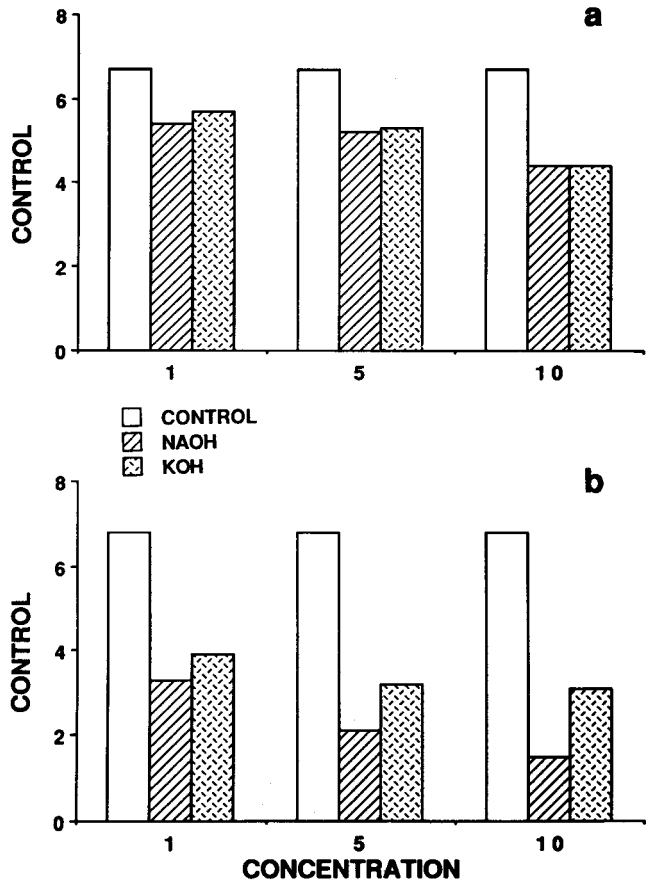

Figure 4. *Effect of NaOH and KOH on* S. marcescens *attached to lean and fat tissues (log CFU/2 cm²). Concentration expressed as percent. (a) lean tissue (b) fat tissue.*

With the low inoculum, there was significant difference between the 1% and 5% concentrations, but not between 5% and 10%. The reduction with the lean tissue and low inoculum was greater than the high inoculum, falling in to the 2 to 3 log range. The reductions on the fat tissue were similar to those seen with the high inoculum.

Staphylococcus aureus: The effects of NaOH and KOH on *S. aureus* are shown in Figure 3. With an average inoculum level of 10^6, there was a significant difference between the two types of tissues. The 10% concentrations reduced the bacterial counts approximately 1 log cycle on the lean tissue and 1.5 to 2.5 logs on the fat tissue. There was a significant difference between 1% and 5%, but not between 5% and 10%. NaOH removed or destroyed significantly more cells than KOH with fat tissue at the 5% and 10% concentrations.

Serratia marcescens: The results of the washing experiments with *S. marcescens* are shown in Figure 4. As with the other bacteria in this study, there was a significant difference between the effects on fat and lean tissue. The effects, however, were more pronounced, particularly with the fat tissue. There was a significant difference between all three concentrations, with the 10% concentration removing or destroying approximately 2 log cycles with the lean tissue and 4 to 5 logs with the fat tissue. There was significant ($P<0.10$) compound by tissue interaction, with NaOH being more effective at 5% and 10% on fat tissue than KOH.

DISCUSSION

The effects of the buffer, ethanol, and NaCl solutions on the attached bacteria were minor. Thomas and McMeekin *(22)* had reported that 0.9% saline prevented the firm attachment of salmonellae to poultry muscle and that rinsing with the same concentration of saline removed many of the attached cells. However, this research was based on direct observation with electron microscopy. Lillard *(14)* was unable to duplicate the results using conventional microbiological methods (i.e. plating) and demonstrated that most of the bacteria were in fact hidden in crevices on the surface of the muscle and, therefore, not visible by electron microscopy. The results presented in this paper agree with the findings of Lillard, indicating that even high concentrations of NaCl have relatively little effect on the detachment of bacteria used in this experiment from muscle surfaces.

Both NaOH and KOH were effective in destroying and/or removing bacteria from the surface of the muscle and fat tissues although the Gram-positive bacteria were more resistant to the bacteriocidal effects. This may be attributable to differences in the cell wall structure. For every bacterial species studied, there was a greater reduction in numbers on fat tissue than on lean tissue. This difference could be attributed to the bacteria being more protected from the toxic effects of the compounds on muscle tissue than on fat or that saponification of fat might make the bacteria physically more accessible to the sanitizer. It is likely that the reduction in numbers on muscle tissue is due to the bacteriocidal action of the compounds, since the results for muscle tissue are similar to those reported by Humphrey et al. *(12)*, who reported the reduction of total aerobic bacteria and "coli-aerogenes" in poultry scald water treated with NaOH. The poultry scald water contained high levels of organic material, which may have provided some protective effect for the bacteria. The difference between the reduction on muscle tissue and the reduction on fat tissue may also be partially attributed to enhanced physical removal of the bacteria by saponification of the fat in addition to the bactericidal effects.

While effective from a microbiological perspective, the higher concentrations (5% & 10%) of NaOH and KOH are extreme treatments. To be useful on meat intended for human consumption, the alkalis would have to be neutralized and rinsed off the meat surface. In addition, there was some discoloration (greying) of the lean tissue at these concentrations. Further studies would be required to determine if this was a temporary or permanent color shift. This discoloration appeared to be on the surface of the meat, and probably would not affect the deep muscle tissue on a carcass.

When the treatment effects are considered in light of the D values, it is obvious that both muscle and fat tissue

provide considerable protection for the attached bacteria. With the exception of the 1% concentration of alkali with *S. aureus* on both tissues and *L. monocytogenes* on fat tissue, all of the treatment effects were substantially less than the effects which might be anticipated from the D values in solution. The differences between the predicted and observed effect for the two exceptions indicated were relatively minor. The nature of the observed protective effect requires further investigation.

ACKNOWLEDGMENT

The author thanks Ms. Terri Alberts and Ms. Tammy Stuehm for their assistance in the laboratory.

REFERENCES

1. Acuff, G. R., C. Vanderzant, J. W. Savell, D. K. Jones, D. B. Griffin, and J. G. Ehlers. 1987. Effect of acid decontamination of beef subprimal cuts on the microbiological and sensory characteristics of steaks. Meat Sci. 19:217-226.
2. Anderson, M. E., H. E., Huff, H. D. Naumann, R. T. Marshall, J. M. Damare, M. Pratt, and R. Johnston. 1987. Evaluation of an automated beef carcass washing and sanitizing system under production conditions. J. Food Prot. 50:562-566.
3. Anderson, M. E., R. T. Marshall, W. C. Stringer, and H. D. Naumann. 1979. Microbial growth on plate beef during extended storage after washing and sanitizing. J. Food Prot. 42:389-392.
4. Ayres, J. C. 1955. Microbiological implications in the handling, slaughtering, and dressing of meat animals. Adv. Food. Res. 6:109-161.
5. Bracewell, A. J., J. O. Reagan, J. A. Carpenter, and L. C. Blankenship. 1985. Incidence of *Campylobacter jejuni/coli* on pork carcasses in the northeast Georgia area. J. Food Prot. 48:808-810.
6. Chandran, S. K., J. W. Savell, D. B. Griffin, and C. Vanderzant. 1986. Effect of slaughter-dressing, fabrication and storage conditions on the microbiological and sensory characteristics of vacuum-packaged beef steaks. J. Food Sci. 51:37-39.
7. Cottin, J., H. Genthon, C. Bizon, and B. Carbonnelle. 1985. Study of *Listeria monocytogenes* in meat taken from 514 cattle. Sci. Aliments 5(series IV):145-149.
8. Crouse, J. D., M. E. Anderson, and H. D. Naumann. 1987. Microbial decontamination and weight of carcass beef as affected by automated washing pressure and length of time of spray. Proc. of the 33rd. Int'l. Congress of Meat Science and Technol., Helsinki, Finland. Vol. 1, pp.70-72.
9. Currier, M., M. Singleton, J. Lee, and D. R. Lee. 1986. *Salmonella* in swine at slaughter: incidence and serovar distribution at different seasons. J. Food Prot. 49:366-368.
10. Doyle, M. P., and J. L. Schoeni. 1987. Isolation of *Escherichia coli 0157:H7* from retail fresh meats and poultry. Appl. Environ. Microbiol. 53:2394-2396.
11. Food and Drug Administration. 1984. Bacteriological analytical manual, 6th. ed. Association of Official Analytical Chemists, Arlington, VA.
12. Humphrey, T. J., D. G. Lanning, and D. Beresford. 1981. The effect of pH adjustment on the microbiology of chicken scald-tank water with particular reference to the death rate of salmonellas. J. Appl. Bacteriol. 51:517-527.
13. Humphrey, T. J., D. G. Lanning, and D. Leeper. 1984. The influence of scald water pH on the death rates of *Salmonella typhimurium* and other bacteria attached to chicken skin. J. Appl. Bacteriol. 57:355-359.
14. Lillard, H. S. 1987. Effect of surfactant or changes in ionic strength on the attachment of *Salmonella typhimurium* to poultry skin and muscle. J. Food Prot. (In press).
15. Lillard, H. S., L. C. Blankenship, J. A. Dickens, S. E. Craven, and A. D. Shackelford. 1987. Effect of acetic acid on the microbiological quality of scalded picked and unpicked broiler carcasses J. Food Prot. 50:112-114.
16. Notermans, S., and E. H. Kampelmacher. 1974. Attachment of some bacterial strains to the skin of broiler chickens. Brit. Poultry Sci. 15:573-585.
17. Ockerman, H. W., R. J. Borton, V. R. Cahill, N. A. Parrett, and H. D. Hoffman. 1974. Use of acetic and lactic acid to control the quantity of microorganisms on lamb carcasses. J. Milk Food Technol. 37:203-204.
18. SAS. 1982. User's guide: Statistics, 1982 ed. SAS Institute Inc., Cary, N.C.
19. Snijders, J. M. A., J. G. van Logtestijn, D. A. A. Mossel, and F. J. M. Smulders, 1985. Lactic acid as a decontaminant in slaughter and processing procedures. Veterinary Quart. 7:277-282.
20. Speck, M. L. (ed.). 1984. Compendium of methods for the microbiological examination of foods, 2nd. ed. American Public Health Association, Washington, D.C.
21. Stevenson, K. E., R. A. Merkel, and H. C. Lee. 1978. Effects of chilling rate, carcass fatness and chlorine spray on microbiological quality and case-life of beef. J. Food Sci. 43:849-852.
22. Thomas, C. J., and T. A. McMeekin. 1981. Attachment of *Salmonella spp.* to chicken muscle surfaces. Appl. Environ. Microbiol. 42:130-134.
23. Woolthuis, C. H. J., and F. J. M. Smulders. 1985. Microbial decontamination of calf carcasses by lactic acid sprays. J. Food Prot. 48:832-837.