ISSN 1682-8356
ansinet.org/ijps

INTERNATIONAL JOURNAL OF

POULTRY SCIENCE

ANSInet
308 Lasani Town, Sargodha Road, Faisalabad - Pakistan
Mob: +92 300 3008585, Fax: +92 41 8815544
E-mail: editorijps@gmail.com

International Journal of Poultry Science 4 (12): 944-946, 2005
ISSN 1682-8356

External Treatment of Broiler Chickens with Lactic-Acid-Producing Bacteria Before Slaughter[1]

J.A. Cason, R.J. Buhr, A. Hinton, Jr., M.E. Berrang and N.A. Cox
USDA/ARS, Russell Research Center, P.O. Box 5677, Athens, GA 30604-5677, USA

Abstract: Lactic-acid-producing bacterial cultures were applied to the skin of live broilers 24 hours before slaughter to determine whether inoculation with the cultures could affect the numbers of bacteria that are normally found on the skin of processed broiler carcasses. The cultures contained 10,000 to 100,000 cfu/mL and were suspended in 250 mL of a pH 6.0 nutrient medium (including glucose, peptone, beef extract, yeast extract, a surfactant, and salts) intended to enhance the survival and growth of the cultures. With broilers suspended by the feet, feathers were moved aside and the liquid suspension was sprayed directly on the skin. Sprayed broilers were then returned to a pen. In each of three replications, 4 six-wk-old broilers were sprayed and 4 broilers were kept as untreated controls. The following day, broilers were processed in a research processing facility and defeathered carcasses were sampled by rinsing for 1 min in 200 mL of peptone water after removal of heads and feet. Coliforms, *E. coli*, lactic-acid bacteria, and *Campylobacter* in carcass rinses were enumerated by standard methods. After removal of aliquots for plating, the remaining sample volume was enriched to detect *Salmonella*. No differences were found in $\log_{10}$(cfu/mL) of coliforms, *E. coli*, or lactic-acid bacteria between the treated and control carcasses. *Salmonella* bacteria were present on some carcasses, but with no difference between treatments. *Campylobacter* spp. were present in only one replication, so numbers of *Campylobacter* could not be analyzed statistically. Spraying lactic-acid-producing bacteria with nutrients on the skin of live broilers on the day before processing appears to have no effect on numbers of bacteria that are present on the skin after defeathering.

Key words: Lactic acid bacteria, skin, antibacterial treatment, chickens

Introduction

Treatment with lactic acid has been reported to increase the shelf life of poultry legs and thighs (Mountney and O'Malley, 1965) and whole carcasses (Van der Marel *et al*., 1988; Sawaya *et al*., 1995). Lactic acid treatments have also been reported to reduce numbers of pathogenic bacteria such as *Salmonella* (Mulder *et al*., 1987) and improve the safety of treated carcasses (Van der Marel *et al*., 1988). Use of lactic-acid-producing bacteria in making of sausage is well known, but mechanically deboned poultry meat has also been treated with lactic acid bacteria to increase shelf life (Raccach and Baker, 1979). Decontamination of meat with either lactic acid or lactic acid bacteria has been reviewed by Guerrero and Taylor (1994).

Lactic-acid-producing bacteria have also been used in competitive exclusion treatments to affect the microbial ecology of the small intestine (Mead, 2000). Lactobacilli have little effect on *Salmonella* colonization of the ceca (Soerjadi *et al*., 1981; Stavric, 1987), although Soerjadi *et al*. (1981) reported that *Lactobacillus* cultures delivered by gavage reduced the numbers of *Salmonella* in the crop of treated birds by one to two logs. Besides any competitive effects of lactic acid bacteria in the intestinal tract or in the crop, the effect of lactic acid itself has been attributed both to pH and direct chemical effects (Zeitoun and Debevere, 1992).

If colonization of the skin of live chickens with lactic acid bacteria can be encouraged by supplying bacteria isolated from chickens and nutrients to encourage growth, it may be possible to affect the normal bacterial flora and possibly pathogens on broilers arriving at the processing plant. The purpose of this experiment was to determine whether numbers of coliforms, *E. coli*, and *Campylobacter* and incidence of *Salmonella* on defeathered carcasses can be reduced by spraying live cultures of lactic acid bacteria on the skin of broilers 24 hours before slaughter.

Materials and Methods

This work was approved by an Institutional Animal Care and Use Committee. The experiment was performed on three days using broiler chickens grown to six weeks of age on pine shavings in a curtain-sided house typical of those in the local poultry industry. Twenty-four hours before slaughter, half of the birds were held by the feet in a head-down position and sprayed with approximately 250 ml of solution containing lactic acid bacteria and nutrients. Feathers were moved aside as much as possible to deliver the spray directly to the skin, with no spraying of control birds. Birds were wet, but showed no discomfort when returned to their pens.

[1]Mention of trade names or commercial products in this publication is solely for the purpose of providing specific information and does not imply recommendation or endorsement by the U.S. Department of Agriculture.

Bacterial inoculum and nutrient solution: Four to five logs of lactic acid-producing bacteria that had been isolated from the crop of six-week-old broilers were added to the spray solutions 1 to 2 hours before the solution was sprayed on the broilers. On Replication 1 the spray was supplemented with $1.5X10^4$ cfu/ml of an unidentified facultative streptococcus, on Replication 2 the spray was supplemented with 2.0×10^4 cfu/ml of a mixed culture of *Lactobacillus minutus* and *Eubacterium alactolyticum*, and on Replication 3 the spray was supplemented with 1.0×10^5 cfu/ml of *Lactobacillus delbrueckii* ss *bulgaricus*. The bacteria were grown in Bacto Lactobacilli MRS Broth (Sigma Chemical Co., St. Louis, MO 63178 USA) in an anaerobic chamber (Coy Laboratory Products, Inc., Grass Lake, MI 49240 USA) for 24 hours at 35°C. Cultures were serially diluted and added to the spray. The spray solution was composed of the following ingredients on a per liter basis: 75 g of D-(+)-glucose, 10 g of proteose peptone (Becton Dickinson, Sparks, MD 21152 USA), 5 g of beef extract (Sigma Chemical Co., St. Louis, MO 63178 USA), 5 g of yeast extract (Sigma Chemical Co., St. Louis, MO 63178 USA), 1 g of polyoxyethylene-sorbitan monooleate (Tween 80™) (Becton Dickinson, Sparks, MD 21152 USA), 0.5 g of magnesium sulfate heptahydrate, and 0.2 g of manganese sulfate monohydrate. The pH of the solution was adjusted to 6.0 with a solution of 3 parts 1 M glacial acetic acid and 1 part 1M propionic acid, after which the solution was autoclaved at 121°C for 15 min and stored at 4°C until ready for use. This nutrient solution had previously been shown to stimulate growth of naturally occurring lactic acid bacteria, lower the pH, and reduce numbers of *Enterobacteriaceae* and *Salmonella* Typhimurium in the crop of broiler chickens that consumed the solution in their drinking water (Hinton *et al.*, 2000, 2002).

After feed withdrawal of approximately 12 to 14 hours, birds were transported to the laboratory in plastic coops. On each day, 4 birds per treatment were processed with sprayed and control birds alternating on the processing line. Birds were hung in shackles and stunned at 50 VAC (30 mA) for 10 s, then bled in cones for 90 s after both carotid arteries were severed. The cloaca of each carcass was plugged with a tampon to prevent escape of feces during defeathering. Carcasses were scalded 2 min at 57°C and then picked individually in an in-line commercial picker which was sprayed thoroughly with 82°C water between individual carcasses to minimize transfer of bacteria. Previous experiments in which the picker was spray cleaned with hot water between treatments were successful in demonstrating treatment differences in numbers of carcass bacteria despite use of the same picking equipment (Musgrove *et al.*, 1997). After removal of the heads and feet, whole carcass rinses were performed by rinsing carcasses in 200 ml of 0.1% buffered peptone water. The neck was left on the carcass to avoid damage to the crop and escape of contents during carcass rinsing. The pH of carcass rinse solutions was measured immediately.

Enumeration of bacteria in rinsates: Rinsates were analyzed to determine the numbers of lactic acid bacteria, *Escherichia coli*, and coliforms recovered from the processed carcasses, along with incidence of *Salmonella*. Serial dilutions of the rinsates were plated on the appropriate bacteriological medium to enumerate bacteria. Lactic acid bacteria were enumerated on Lactic Acid Bacteria Agar Medium (Atlas, 1993). Plates were incubated anaerobically at 35-37°C. Coliform and *E. coli* counts were made by plating 1 mL from a serial dilution of the samples on duplicate *E. coli* Petrifilm J (3M Health Care, St. Paul, MN 55144-1000 USA) incubated for 48 hours at 35-37°C. *Campylobacter* was enumerated by plating serial dilutions on duplicate Campy-Cefex agar plates (Stern *et al.*, 1992) incubated at 42°C for 48 hours in a microaerobic environment (5% O_2, 10% CO_2, 85% N). For culturing of *Salmonella*, 30 ml of buffered peptone water was added to a 30-ml aliquot from the carcass rinse liquid and the suspension was incubated at 37°C for 24h. After incubation, 0.5 ml of incubated rinse solution was transferred to 10 ml of Rappaport-Vassiliadis broth (unless specified otherwise, microbiological supplies were from Becton Dickinson, Sparks, MD 21152 USA) and to 10 ml of tetrathionate broth (Hajna) and incubated at 42°C for 24 h. Each broth was then streaked onto BG Sulfa agar plates and incubated for 24 hours at 35°C. Suspect *Salmonella* colonies were picked and inoculated onto triple sugar iron agar and lysine iron agar slants and incubated for 24 hours at 35°C. Presumptive isolates were confirmed by Poly O and Poly H (Microgen, Camberly, Surrey, UK) agglutination tests.

Bacterial counts per ml of rinse were converted to log10 for statistical analysis. Analysis of variance was conducted using PROC GLM of SAS (SAS Institute, 2000) to test for differences in numbers of bacteria recovered from the rinses at 0 and 24 hours after chilling. The level for significance was P= 0.05.

Results and Discussion

There were no significant differences between carcasses treated with lactic-acid-producing bacteria before slaughter and untreated control carcasses in numbers of coliforms and *E. coli*, nor was there any difference in numbers of lactic acid bacteria recovered in the carcass rinses (Table 1). Treatment had no effect on incidence of *Salmonella* (16.6% in both treated and control carcasses). *Campylobacter*-positive carcasses were found in only one replication, so the number of observations was too small to reach any conclusion concerning the effect of the treatment on *Campylobacter*. The combination of an acidic nutrient solution and inoculated bacteria had no effect on bacteria of interest on the treated chicken carcasses.

Although it is possible that inoculated bacteria may have replaced some of the naturally occurring bacteria on the skin of treated chickens, the inability of the spray treatment to increase numbers of lactic acid bacteria on the exterior of defeathered carcasses suggests that

Table 1: Means and standard deviations for numbers of coliforms, *E. coli*, and lactic acid bacteria in rinses of control and treated broiler carcasses sprayed with lactic-acid-producing bacteria and nutrients 24 hours before processing on each of 3 replicates. Means were not significantly different

Bacteria	Treatment	Replicate 1	Replicate 2	Replicate 3
		$\log_{10}$(cfu/ml)		
Coliforms	Control	3.4 ± 0.4	3.2 ± 0.6	3.6 ± 0.7
	Sprayed	3.4 ± 0.7	3.3 ± 0.5	3.8 ± 0.9
E. coli	Control	2.6 ± 0.7	2.8 ± 0.4	3.4 ± 0.9
	Sprayed	2.8 ± 1.0	2.9 ± 0.3	3.6 ± 0.9
Lactic-acid- producing bacteria	Control	4.7±0.4	4.4±0.8	5.0±0.5
	Sprayed	4.7 ± 0.7	4.9 ± 0.7	4.9 ± 0.6

inoculated bacteria generally did not survive and grow. The inoculated bacteria had to compete with the normal skin flora and 24 hours may not have been long enough for the inoculated cultures to adjust to conditions and start growing. Lactic acid bacteria generally grow best in anaerobic or microaerobic environments, as in the crop where Soerjadi *et al.* (1981) reported that *Lactobacillus* cultures delivered by gavage reduced the numbers of *Salmonella* by one to two logs. Similarly, Hinton *et al.* (2000) found that numbers of naturally occurring lactic acid bacteria increased and numbers of *Enterobacteriaceae* and *Salmonella* decreased in the crop of chickens consuming the nutrient solution used in the present experiment in their drinking water.

Application of an acidic nutrient solution was intended to provide a more favorable environment for the inoculated bacteria, but the buffering capacity of the skin appears to be high enough to prevent the acidic conditions from lasting. Van der Marel *et al.* (1988) reported reduction of skin surface pH by 3 or 4 units on processed carcasses, but those carcasses were scalded and defeathered before treatment with lactic acid. It seems unlikely that such a large change in pH could occur in the case of birds that were alive when treated. The mean pH of carcass rinses was not different between control and sprayed broilers, with a mean of 7.3 for both. The spray liquid had a pH of 6.0, so any meaningful change in skin pH would have had to come from proliferation of the lactic acid bacteria. If there was a pH effect from the spray or the lactic-acid bacteria, it had disappeared by the time of processing.

References

Atlas, R., 1993. Handbook of Microbiological Media. CRC Press, Boca Raton FL.

Guerrero, I. and A.J. Taylor, 1994. Meat surface decontamination using lactic acid from chemical and microbial sources. Lebensm. Wiss. Tec., 27: 201-209.

Hinton, Jr., A., R.J. Buhr and K.D. Ingram, 2000. Reduction of *Salmonella* in the crop of broiler chickens subjected to feed withdrawal. Poult. Sci., 79: 1566-1570.

Hinton, A. Jr., R.J. Buhr and K.D. Ingram, 2002. Carbohydrate-based cocktails that decrease the populations of *Salmonella* and Campylobacter in the crop of broilers chickens subjected to feed withdrawal. Poult. Sci., 81: 780-784.

Mead, G.C., 2000. Prospects for "competitive exclusion" treatments to control Salmonellas and other foodborne pathogens in poultry. Vet. J., 159: 111-123.

Mountney, G.J. and J.O. Malley, 1965. Acids as poultry meat preservatives. Poult. Sci., 44: 582-586.

Mulder, R.W.A.W., M.C. van der Hulst and N.M. Bolder, 1987. *Salmonella* decontamination of broiler carcasses with lactic acid, L-cysteine, and hydrogen peroxide. Poult. Sci., 66: 1555-1557.

Musgrove, M.T., J.A. Cason, D.L. Fletcher, N.J. Stern, N.A. Cox and J.S. Bailey, 1997. Effect of cloacal plugging on microbial recovery from partially processed broilers. Poult. Sci., 76: 530-533.

Raccach, M. and R.C. Baker, 1979. The effect of lactic acid bacteria on some properties of mechanically deboned poultry meat. Poult. Sci., 58: 144-147.

SAS Institute, 2000. SAS/STAT7 User's Guide. Version 8. SAS Institute Inc., Cary, NC.

Sawaya, W.N., A.S. Elnawawy, S. Al-Zenki, J. Al-Otaibi, H. Al-Omirah and H. Al-Amiri, 1995. Storage stability of chicken as affected by MAP and lactic acid treatment. J. Food Sci., 60: 611-614.

Soerjadi, A.S., S.M. Stehman, G.H. Snoeyenbos. O.M. Weinack and C.F. Smyser, 1981. The influence of lactobacilli on the competitive exclusion of paratyphoid salmonellae in chickens. Avian Dis., 25: 1027-1033.

Stavric, S., 1987. Microbial colonization control of chicken intestine using defined cultures. Food. Tec., 41: 93-98.

Stern, N.J., B. Wojton and K. Kwiatek, 1992. A differential selective medium and dry ice generated atmosphere for recovery of *Campylobacter jejuni*. J. Food Prot., 46: 339-344.

Van der Marel, G.M., J.G. van Logtestjin and D.A.A. Mossel, 1988. Bacteriological quality of broiler carcasses as affected by in-plant lactic acid decontamination. Int. J. Food Microbiol., 6: 31-42.

Zeitoun, A.A.M., and J.M. Debevere, 1992. Decontamination with lactic acid/sodium lactate buffer in combination with modified atmosphere packaging effects on the shelf life of fresh poultry. Int. J. Food Microbiol., 16: 89-98.