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Full Length Research Paper

Impact of feed withdrawal and addition of acetic acid in drinking water during preslaughter phase on intestinal microbiota of broilers

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This study investigated the impacts of feed withdrawal and addition of acetic acid in drinking water on the pH and microflora of gizzard, cecal and feces in preslaughter broiler chickens. Twenty four (24) individually caged 42 days old male Ross 308 broilers with almost equal weight were randomly divided into six treatments with four replicates each. The control group had free access to feed and water, but without supplemental acetic acid during preslaughter period. Another group was kept 8 h without feed, but with *ad libitum* access to unsupplemented drinking water. The other four treatment groups received 1.5, 3, 4.5 or 6% acetic acid added to their drinking water with feed withdrawal for 8 h. Fecal samples were collected 1 h before slaughter. After slaughter, gizzard and cecal contents were collected for microbiological studies. The number of *Clostridium*, *Bacillus*, *Coliform* and other bacteria were enumerated on appropriate bacterial media. The pH of gizzard and feces was significantly (P<0.05) lower in birds that underwent feed withdrawal combined with acidified water as compared with the control and feed withdrawal groups without acetic acid supplementation. The cecal pH was significantly lower for the 4.5 and 6% acetic acid groups compared to the control and feed withdrawal group with no acid. Also, the addition of acetic acid to drinking water resulted in significant (P<0.05) reduction of *Bacillus, Clostridium, Coliform*, facultative aerobic, and other bacteria in gizzard, cecal and fecal contents of birds with acidified water in comparison to the control and feed withdrawal treatments. Under the condition of this trial, addition of acetic acid in the drinking water 8 h before slaughter could reduce the concentration of certain populations of bacteria and might be a suitable approach against bacterial contamination of broiler carcass during processing.

Key words: Acetic acid, feed withdrawal, *Bacillus* and *Clostridium*, broilers.

INTRODUCTION

Bacterial contamination of poultry products continues to be a major concern for consumers. Moreover, feed withdrawal before transport of the birds to abattoir is common to avoid contamination of the carcasses with excreta during slaughter and processing. However, it has been reported that intestinal tissues from fasted birds are more susceptible to pathogen attachment than tissues from control birds (Burkholder et al., 2008). For this reason, different workers have evaluated suitable and acceptable decontaminant chemicals or processes to reduce or eliminate enteric pathogens from poultry products in recent years (Antunes et al., 2003; Luckstadt, 2007). On the other hand, a vast variety of bactericides such as antibiotics have been assessed for their efficacy to reduce microbial loads on poultry carcasses. Due to increasing pressures of antibiotic resistance and food

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safety concerns, the use of their alternatives in livestock and poultry feeds is becoming more common in recent years. Organic acids are routinely included in diets for monogastric animals in Europe as preservative and acidifier, in order to replace antibiotics as growth promoters and prevent or control pathogens (Papatsiros et al., 2012). For instance, Saki et al. (2012) demonstrated that organic acids significantly reduced Enterobacteriaceae counts in ileum and cecum of broiler chickens at 21 and 42 days of age.

Various organic acids which are particularly effective against acid-intolerant species such as *Escherichia coli, Salmonella* and *Campylobacter* have been used in feed by different researchers (Izat et al., 1990a; Luckstadt, 2007; Thompson and Hinton, 1997). Also, it has been indicated that acidified drinking water significantly prevented the *Campylobacter* spread via drinking water in broiler flocks (Chaveerach et al., 2004). Interestingly, addition of organic acids to the drinking water before slaughter might reduce crop *Salmonella enteritidis* colonization and can be an approach to reduce *Salmonella* contamination of broiler products during processing (Avila et al., 2003). Thus, the main aim of the current research was to evaluate the effect of feed withdrawal and administration of acetic acid in drinking water during preslaughter on pH and intestinal microflora of broiler chickens.

MATERIALS AND METHODS

A total of 24 Ross 308 male broiler chickens at the age of 42 days were used in this study. Before commence of the trial, the birds were transported from farm house to experimental house. The chickens were kept in individual crates. These birds were allocated to six treatments with four replicates each. This trial was planned as completely randomized design during a period of 8 h before slaughter.

Control group had free access to feed and water, but without supplemental acetic acid during preslaughter period. Another group was kept 8 h without feed, but with *ad libitum* access to unsupplemented drinking water before slaughter. For the other treatments, four levels of acetic acid were added to the drinking water (1.5, 3, 4.5, and 6%) of broilers with preslaughter feed withdrawal.

Fecal samples were collected 1 h before slaughter via cloacae swab. Then, all chickens were killed by cervical dislocation. After slaughter, gizzard and cecal contents were collected for microbiological studies. All samples were kept in -20°C until analyses in the laboratory. The samples were homogenized, and then 1 g of each sample was collected and transferred into 10 ml sterile saline solution for dilution. The pH of the homogenized contents of gizzard, cecal and feces was measured using pH meter (Model 691, Metrohm, Switzerland) just after sampling for serial dilution. After that, each sample was spread on selective agar plates as follows. Aerobic plate count was used for the total aerobic bacteria. Eosin methylene blue agar (EMB) was utilized for coliform. Also, thio glycolate media (TGA) was used for *bacillus* and *clostridium* bacteria (APHA, 1993). After incubation at 37°C for 48 h under micoareophilic conditions, the number of bacterial colonies was determined.

Primary, data of microflora number from gizzard, cecal and feces were converted to $\log_{10}$ per ml (CFU) before the analysis. All data were analyzed using the General Linear Model procedure of the Statistical Analysis System (SAS, 2004). Duncan's multiple range test was used to compare the means. All statements of significance were based on probability of $P<0.05$.

RESULTS AND DISCUSSION

The addition of acetic acid to drinking water 8 h before slaughter caused a lower pH as is presented in Table 1. The pH of gizzard, cecal and feces was significantly ($P<0.05$) lower in birds that drank acidified water, particularly in 4.5 and 6% acetic acid as compared with the control and feed withdrawal groups (Table 1).

These results are in agreement with Byrd et al. (2001) who reported reduced pH of crop in birds treated with 0.5% acetic acid, 0.5% lactic acid and 0.5% formic acid for 8 h during preslaughter feed withdrawal. Diminution of gastrointestinal pH has adverse impacts for colonization of acid-intolerant bacteria such as *Coliforms* (Luckstadt, 2007). Nevertheless, our results contrast with the findings of Watkins et al. (2004) who demonstrated that acidified water by using acetic acids did not cause significant reduction in the gizzard pH of broilers. The discrepancies in these results might be due to differences in the type and concentration of organic acid used in the studies.

The incorporation of acetic acid into drinking water was associated with a significant reduction in *Bacillus, Clostridium, Coliform*, facultative aerobic, and other bacteria of gizzard, ceca, and feces in acidified water treatments as compared with the control and feed withdrawal treatments (Tables 2, 3 and 4). These results are in line with Philipsen (2006) that revealed that addition of organic acid to the drinking water helps to reduce the level of pathogens in the water and to regulate gut microflora. Moreover, Moharrery and Mahzonieh (2005) observed that the addition of 0.1% malic acid to drinking water significantly reduced *E. coli* counts in the small intestine of layer chickens. As well, Chaveerach et al. (2004) reported decreased numbers of *Campylobacter* in the cecal contents of birds which consumed acidified water. Furthermore, Bryd et al. (2001) and De Avila et al. (2003) suggested that incorporation of some organic acids in the drinking water during the preslaughter feed withdrawal period significantly reduced *Salmonella* and *Campylobacter* contamination of crops and broiler carcasses at processing. In contrast to the above mentioned results, Aciokgoz et al. (2011) reported that formic acid did not significantly change the microflora of broilers exposed to heat stress that might be due to a more hygienic evisceration process or lower microbial load in the gastrointestinal tract.

Organic acids have an antimicrobial effect because they diffuse through the bacterial cell membrane, and then dissociate into anions and protons, and eventually disturb the intracellular electron-balance (Luckstadt, 2007; Strauss and Hayler, 2001). Organic acids have also been shown to be effective in lowering some bacterial numbers recovered from poultry carcasses when

Table 1. The pH of gizzard and cecal contents and feces in broiler chickens at 42 days of age.

Treatment	Gizzard	Ceca	Feces
Free access to feed and untreated water	3.80[a]	6.85[a]	6.87[a]
Feed withdrawal with untreated water	3.25[b]	6.95[a]	6.95[a]
Feed withdrawal with acetic acid in water (%)			
1.5	3.10[c]	6.95[a]	6.05[b]
3	2.95[d]	6.80[a]	6.17[b]
4.5	2.80[d]	6.42[b]	5.75[c]
6	2.60[d]	6.40[b]	5.97[c]
±SEM	0.148	0.162	0.195

[abcd]Means with different superscripts in each column are significantly different (P<0.05).

Table 2. The gizzard microbial population (log $_{10}$ cfu) of broiler chickens at 42 days of age.

Treatment	*Bacillus* and *Clostridium*	*Coliform*	Facultative aerobic bacteria	Other bacteria
Free access to feed and untreated water	5.04[c]	4.97[b]	5.33[b]	4.01[a]
Feed withdrawal with untreated water	5.28[a]	5.16[a]	5.54[a]	4.09[a]
Feed withdrawal with acetic acid in water (%)				
1.5	5.33[a]	4.70[c]	5.43[b]	3.38[b]
3	5.14[c]	4.61[c]	5.26[c]	3.07[c]
4.5	5.06[c]	4.27[d]	5.15[c]	3.91[a]
6	5.23[b]	4.22[d]	5.28[c]	3.52[b]
±SEM	0.023	0.041	0.019	0.074

[abcd]Means with different superscripts in each column are significantly different (P<0.05).

Table 3. The cecal microbial population (Log $_{10}$ cfu) of broiler chickens at 42 days of age.

Treatment	*Bacillus* and *Clostridium*	*Coliform*	Facultative aerobic bacteria	Other bacteria
Free access to feed and untreated water	5.31[a]	5.89[ab]	6.07[a]	5.26[a]
Feed withdrawal with untreated water	5.31[a]	5.97[a]	6.10[a]	5.30[b]
Feed withdrawal with acetic acid in water (%)				
1.5	5.24[b]	5.81[bc]	5.98[b]	5.18[b]
3	5.05[c]	5.77[c]	5.90[c]	5.15[b]
4.5	5.07[c]	5.67[c]	5.82[d]	5.12[bc]
6	5.23[b]	5.73[c]	5.90[c]	5.10[c]
±SEM	0.018	0.033	0.023	0.023

[abcd]Means with different superscripts in each column are significantly different (P<0.05).

used in scald water (Izat et al., 1990b). Likewise, it has been indicated that acidifiers could diminish *E. coli, Coliform*, and *Clostridium perfringens* in the gut of Japanese quail (Ghosh et al., 2007), or reduce *Salmonella* in carcass and feces of broilers (Patten and Waldroup, 1988) and decrease the microbial contamination of hatchery spoilage in broiler breeder (Humphrey and Lanning, 1988).

In conclusion, the addition of acetic acid in drinking water 8 h before slaughter might help to reduce gizzard, ceca and fecal contamination by pathogenic bacteria and reduce microbial loads on poultry carcasses. However, it should be taken into account that high concentration of acetic acid (6%) in comparison to 4.5% inclusion level caused a significant increase of *Coliform* and other bacteria in feces (Table 4). This phenomenon might indicate that a kind of resistance to overuse of acetic acid has been created. Future studies could examine other

Table 4. The fecal microbial population (Log $_{10}$ cfu) of broiler chickens at 42 days of age.

Treatment	*Bacillus* and *Clostridium*	*Coliform*	Facultative aerobic bacteria	Other bacteria
Free access to feed and untreated water	5.48[a]	4.84[ab]	5.90[a]	5.63[a]
Feed withdrawal with untreated water	5.50[a]	4.96[a]	5.77[b]	5.24[b]
Feed withdrawal with acetic acid in water (%)				
1.5	5.38[b]	4.52[c]	5.55[c]	4.88[cd]
3	5.29[c]	4.82[b]	5.52[c]	4.86[cd]
4.5	5.04[d]	4.54[c]	5.50[c]	4.07[d]
6	5.10[d]	4.98[a]	5.46[d]	4.98[c]
±SEM	0.021	0.046	0.018	0.035

[abcd]Means with different superscripts in each column are significantly different (P<0.05).

organic acids to determine the optimum level for the control of certain pathogens.

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