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Full Length Research Paper

Alteration of gut microflora through citric acid treated drinking water in preslaughter male broilers

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This experiment was conducted to evaluate feed withdrawal and addition of citric acid in drinking water on the pH and microflora of gizzard, ceca and feces in preslaughter broiler chickens. A total of 24 male Ross 308 broiler chickens at the age of 42 days with almost equal weight were randomly divided into six treatments with four replicates each. Control group had free access to feed and water, but without supplemental citric acid during preslaughter period. Another group was kept 8 h without feed, but with *ad libitum* access to unsupplemented drinking water. For other treatments, four concentrations of citric acids were added to the drinking water (1.5, 3, 4.5, and 6%) of broilers with feed withdrawal for 8 h. Fecal samples were collected 1 h before slaughter. After slaughter, gizzard and cecal contents were obtained and used for microbiological studies. The number of clostridium, bacillus, coliform and other bacteria were enumerated on appropriate bacterial media. The pH of gizzard, ceca and feces was significantly (P<0.05) lessened in birds that drank acidified water particularly at 4.5 and 6% citric acid as compared with control and feed withdrawal groups without citric acid supplementation. Also, the addition of citric 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 study, addition of citric acid in the drinking water 8 h before slaughter could reduce intestinal microflora colonization and might be a fruitful strategy against bacterial contamination of broiler products during processing.

Key words: Citric acid, feed withdrawal, intestinal bacteria, broiler.

INTRODUCTION

Microorganisms in alimentary tract of animals are small players of the great games as diverse as digestion and diseases. Nowadays, various approaches such as biotechnological procedures (Esmaeilzadeh et al., 2012) and nutritional modifications (Jahani-Azizabadi et al., 2011) have been appraised for special microbiological aims in animal husbandry. In spite of the above mentioned advances, bacterial contamination of poultry products remained to be of great concern for consumers.

Moreover, feed withdrawal before transport of the birds to abattoir is common to avoid contamination of the carcass 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 have been assessed for their efficacy to reduce microbial loads on poultry carcasses.

Various organic acids which are particularly effective

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

Treatment	Gizzard	Ceca	Feces
Free access to feed and untreated water	3.80[a]	6.85[b]	6.87[a]
Feed withdrawal with untreated water	3.25[b]	6.95[b]	6.95[a]
Feed withdrawal with citric acid in water (%)			
1.5	2.42[c]	7.15[a]	6.32[b]
3	2.30[d]	6.80[b]	5.25[c]
4.5	2.15[e]	6.40[c]	6.25[c]
6	2.15[e]	6.45[c]	6.22[c]
±SEM	0.144	0.160	0.180

[a, b, c ,d, e] Means with different superscripts in each column are significantly different (P<0.05).

against acid-intolerant species such as *E. coli,* Salmonella and Campylobacter have been used through feed by different researchers (Izat et al., 1990a; Luckstadt, 2007; Thompson and Hinton, 1997). Furthermore, 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 in 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 objective of current trial was to evaluate the effects of feed withdrawal and administration of citric acid in drinking water during preslaughter period 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 citric 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 concentrations of citric 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. Then, all chickens were killed by cervical dislocation. After slaughter, gizzard and cecal contents were subjected to microbiological studies. All samples were kept at -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, ceca and fecal was measured using pH meter 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. Thio glycolate medium (TGA) was used for bacillus and clostridium bacteria (APHA, 1993). The number of bacterial colonies was recorded after incubation at 37°C for 48 h under micoareophilic conditions.

Number of microflora in gizzard, ceca and feces was converted to $\log_{10}$ per ml before statistical 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 effects of experimental treatments on pH of gizzard, ceca and feces are presented in Table 1. The pH of gizzard, ceca and feces were significantly (P<0.05) lower in birds that drank acidified water, particularly at 4.5 and 6% citric acid as compared with the control and feed withdrawal groups. These results are in agreement with Byrd et al. (2001) which reported that the pH of crop was reduced in birds offered 0.5% acetic acid, 0.5% lactic acid and 0.5% formic acid treatments for 8 h during preslaughter feed withdrawal. Reduction of gastrointestinal pH has adverse impacts for colonization of acid intolerant bacteria such as Coliforms (Luckstadt, 2007). Nevertheless, our results contradict the findings of Watkins et al. (2004) that demonstrated that acidified water 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 in the studies.

The incorporation of citric acid specially at 4.5 and 6% inclusion levels into drinking water significantly declined 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 those of Philipsen (2006) that revealed that the 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%

Table 2. The gizzard microbial population (Log $_{10}$ cfu) of broiler chickens at 42 day 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 citric acid in water (%)				
1.5	5.20[b]	4.20[c]	5.25[c]	3.76[b]
3	5.19[b]	4.13[c]	5.24[c]	3.62[c]
4.5	5.188[b]	4.17[c]	5.23[c]	3.30[d]
6	5.09[c]	3.95[d]	5.13[d]	3.28[d]
±SEM	0.019	0.040	0.014	0.044

[a, b, c, d] 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 day of age.

Treatment	Bacillus and clostridium	Coliform	Facultative aerobic bacteria	Other bacteria
Free access to feed and untreated water	5.30[a]	5.89[ab]	6.06[a]	5.25[a]
Feed withdrawal with untreated water	5.30[a]	5.96[a]	6.69[a]	5.05[b]
Feed withdrawal with citric acid in water (%)				
1.5	5.17[a]	5.85[b]	5.97[b]	4.98[c]
3	4.76[b]	5.71[c]	5.82[c]	4.93[c]
4.5	4.90[b]	5.49[d]	5.67[d]	4.86[d]
6	4.84[b]	5.49[d]	5.67[d]	4.77[e]
±SEM	0.089	0.033	0.024	0.021

[a,b,c,d,e] Means with different superscripts in each column are significantly different (P<0.05).

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

Treatments	Bacillus and Clostridium	Coliform	Facultative aerobic bacteria	Other bacteria
Free access to feed and untreated water	5.47[a]	4.83[ab]	5.90[a]	5.62[a]
Feed withdrawal with untreated water	5.50[a]	4.95[a]	5.76[b]	5.24[b]
Feed withdrawal with citric acid in water (%)				
1.5	5.36[b]	4.52[d]	5.48[c]	4.56[c]
3	5.30[c]	4.71[bc]	5.46[c]	4.55[c]
4.5	5.26[c]	4.59[c]	5.41[d]	4.53[c]
6	5.18[d]	4.55[cd]	5.36[e]	4.62[c]
±SEM	0.101	0.163	0.091	0.160

[a,b,c ,d,e] Means with different superscripts in each column are significantly different (P<0.05).

malic acid to drinking water significantly reduced E. coli counts in the small intestine of laying hens. Chaveerach et al. (2004) reported the 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 used in scald water (Izat et al., 1990b). Likewise, it has been indicated that acidifiers could decrease *E. coli*, Coliforms, 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 citric acid particularly at the levels of 4.5 or 6% in drinking water 8 h before slaughter might help to reduce gizzard, cecal and fecal contamination by pathogenic bacteria and reduce microbial loads on poultry carcasses. In addition, it is noteworthy to conduct more experiments for the evaluation of other organic acids and compare with these results to find the best acidifier for poultry industry.

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