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Effects of Formic Acid Administration in the Drinking Water on Performance, Intestinal Microflora and Carcass Contamination in Male Broilers under High Ambient Temperature

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ABSTRACT : In this study, we examined the effects of formic acid administration to the drinking water on performance, intestinal microflora and carcass contamination in male broilers. A total of 312 day-old male broiler chicks were allocated to two groups with three replicates. The first group (control) received normal drinking water (pH 7.4) during the experiment. The second group consumed acidified drinking water (pH 4.5) after 5 d of age. At 43 d of age, twelve birds were randomly selected from the control group to determine the effect of acidified drinking water on carcass contamination. These birds were only given normal or acidified (pH 3) drinking water for 8 h prior to slaughter. The reduction of water pH from 7.4 to 4.5 significantly decreased body weights of male broilers at 21 and 42 d of age. However, no differences were observed between male broilers given normal and acidified drinking water in terms of feed intake, feed conversion ratio and mortality. The pH value of the gizzard contents was not significantly affected by acid water treatment. There were no significant differences in the intestinal population of *E. coli*, *total organism* and *Salmonella* between the groups. The *total organism* and *E. coli* counts of the carcass slightly decreased in the acidified group. No *Salmonella* was identified in carcass samples of any of the treatment groups. The results showed that drinking water acidification did not provide beneficial effects on performance, intestinal microflora and carcass contamination in male broilers. (**Key Words :** Broiler, Water, Formic Acid, Performance, Intestinal Microflora, Carcass Contamination)

INTRODUCTION

Organic acids have been used for more than 30 years to prevent the bacterial and fungal destruction of feedstuffs (Giesen, 2005; Freitag, 2007). Besides, they are utilized as an alternative for antibiotic growth promoters (AGPs) in animal nutrition (Guathier, 2005; Hernández et al., 2006; Steiner, 2006), since using antibiotics in diets has been banned as a result of increasing pressure from consumers, due to the negative effects on human health.

Currently, drinking water acidification is another implementation in the broiler industry used for improving performance (Cornelison et al., 2005). Addition of organic acid to the drinking water helps to reduce the level of pathogens in the water and the crop/proventriculus, to regulate gut microflora, to increase the digestion of feed and to improve growth performance (Philipsen, 2006). Desai et al. (2007) indicated that inclusion of a combination of formic and propionic acids in the drinking water increased weight gain and improved feed conversion ratio in broilers, which they considered was the cause of higher nitrogen retention. In addition, Samanta et al. (2010) reported that organic acids raised gastric proteolysis and improved the digestibility of protein and amino acids. According to Van Der Sluis (2002), the positive effect of organic acids on digestion was related to a slower passage of feed in the intestinal tract, a better absorption of the necessary nutrients and less wet droppings.

On the other hand, improvements in growth performance are frequently attributed to the composition and activity of the gut microflora which affects nutrient utilisation (Yang et al., 2009). Organic acids have an antimicrobial effect because they diffuse through the bacterial cell membrane, and then dissociate into anions and protons thus disturbing the electron-balance inside the cell (Philipsen, 2006). Several studies have also reported that both dietary formic and propionic acids reduce *Salmonella* and *E. coli* in the small intestinal, cecal, and fecal contents of chickens (Izat et al., 1990a, 1990b; Al-Tarazi and Alshawabkeh, 2003). Moharrery and Mahzonieh (2005)

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observed that the addition of 0.1% malic acid to water significantly reduced *E. coli* counts in the small intestine of layer chickens. In addition, Chaveerach et al. (2004) reported decreased numbers of *Campylobacter* in the cecal contents of birds which consumed acidified water.

Feed is usually withdrawn for several hours before slaughter in order to reduce the potential for carcass contamination from the crop and intestinal contents (Bilgili, 2002). However, feed withdrawal may cause a decrease in lactic acid concentration of the crop which may also be accompanied by an increase in the crop pH and in *Salmonella* crop contamination (Corrier et al., 1999a). Nevertheless, the incidence of *Salmonella* crop contamination might be increased up to fivefold during feed withdrawal and is probably caused by coprophagy (Corrier et al., 1999b). Bryd et al. (2001) and De Avila et al. (2003) suggested that incorporation of some organic acids in the drinking water during the pre-slaughter feed withdrawal period significantly reduced *Salmonella* and *Campylobacter* contamination of crops and broiler carcasses at processing.

High ambient temperature causes significant economic losses in the broiler industry owing to decreased body weight, poor feed conversion ratio and increasing mortality. Heat-stress leads to panting, decreases the partial pressure of $C0_2$ in blood and causes respiratory alkalosis (Bottje and Harrison, 1985; Teeter et al., 1985). Therefore, acidifiers have been used to alleviate negative effects of heat stress and to improve broiler performance by altering acid-base balance. Furthermore, acidified water is expected to be more effective than dietary acidification, since organic acid intake is decreased depending on the reduction in feed consumption during heat stress (Daskiran et al., 2004).

In the broiler industry, different organic acids have been used in the drinking water. Formic, acetic and propionic acids have very good solubilities in water (Freitag, 2007). However, propionic and acetic acid might cause bad taste, blocking of waterlines and nipples by virtue of slime formation thereby reducing water intake (Pilipsen, 2006). Especially, laboratory trials have proven that formic acid singly and its combinations have higher bactericidal effect on *E. coli* and *Salmonella* (Liem, 2007; Stonerock, 2007). Therefore, in our study we preferred using formic acid. The objective of the present study was to determine the effects of formic acid administration in the drinking water on performance and intestinal microflora of male broilers under high ambient temperature. We further examined the effect of acid water treatment during the pre-slaughter fasting period on carcass contamination.

MATERIAL AND METHODS

Experimental design and measurements

This experiment was maintained in an open-sided house. A total of 312 Ross-308 male broiler chicks were used. One-day-old chicks were individually weighed, wing banded and randomly distributed into two main groups with three replicates of 52 chicks each. After giving normal drinking water for the first 5 days, the first group (control) continued receiving normal drinking water (pH 7.4) and the second group consumed acidified drinking water (pH 4.5). In the second group, the water pH was adjusted by using formic acid. Water was refreshed every day in both of the experimental groups.

The birds of the two experimental groups were given the same starter (0 to 21 d) and grower (22 to 42 d) diets (Table 1). Feed was continuously offered *ad libitum* and water was supplied *ad libitum* throughout the trial period via a suspended nipple drinker line. The lighting schedule was 24 h of light per day. During the experimental period, the average daily temperature and relative humidity inside

Table 1. The ingredient and chemical compositions of the experimental diets

Ingredients (%)	Starter diet (0-21 d)	Grower diet[1] (22-42 d)
Maize	42.92	55.70
Soybean meal	33.35	23.38
Full-fat soybean	13.90	11.66
Vegetable oil	5.00	5.00
Monocalcium phosphate	1.93	1.52
Limestone	1.67	1.29
DL-methionine	0.38	0.18
Liquid methionine	-	0.16
VitaLys	0.13	0.32
L-Threonine	-	0.07
Salt	0.40	0.40
Coccidiostat[2]	0.07	0.07
Choline chloride	0.05	0.05
Vitamin/mineral premix[3]	0.20	0.20
Chemical composition (%)		
Dry matter	98.54	98.01
Crude protein	24.40	21.17
Ether extract	8.40	7.44
Crude fibre	2.58	1.68
Crude ash	7.51	5.56
Total calcium	1.50	0.90
Total phosphorus	0.83	0.72
ME (MJ/kg)	12.47	12.64

[1] Not supplemented with coccidiostat for the last 5 days.
[2] Supplied per kg of diet: 105 ppm Lasalocid sodium (Avatec, Roche).
[3] Supplied per kg of diet: retinol acetate 5.16 mg; cholecalciferol 0.05 mg; dl-alpha-tocopheryl acetate 30 mg; menadione 5 mg; thiamine 3 mg; riboflavin 8 mg; niacin 25 mg; calcium-D pantothenate 15 mg; pyridoxine 5 mg; cyanocobolamin 0.02 mg; folic acid 1mg; D-biotin 0.05 mg; choline chloride 200 mg; manganese 80 mg; iron 60 mg; zinc, 60 mg; copper 5 mg; cobalt 0.2 mg; iodine 1 mg; selenium 0.15 mg.

the house ranged from 30 to 36°C and 60 to 70%, respectively.

Body weights were also determined individually at 21 and 42 d of age. Feed intake of each replicate was measured from 0 to 21, 22 to 42 and 0 to 42 d. Feed conversion ratio was calculated on the basis of unit feed consumed to unit body weight gain for each replicate separately, taking mortality into consideration.

At 42 d of age, eight males from each group were randomly selected for slaughter. Feed and water were not withdrawn from the birds prior to processing. The birds were slaughtered and manually eviscerated, trying not to rupture the digestive tract segments. The pH values of the gizzard contents were measured using a pH meter (Thermo Scientific Orion-3star Benchtop pH Meter Cat no: 1112000) as described by Chaveerarch et al. (2004). In addition, intestinal contents from six males of each group were evaluated for *total organism*, *E. coli* and *Salmonella* at Bornova Veterinary Control and Research Institute. After slaughter, intestinal contents were immediately collected into sterile tubes in ice and transferred to the laboratory. *Total organism* and *Salmonella* counts were identified on the basis of Turkish Standard Institute Methods (TS, 2004; TS, 2006). *E. coli* counts were detected according to the National Standard Method (2005).

To determine the effect of formic acid administration in the drinking water during pre-slaughter feed withdrawal on microbiological quality of the carcass, twelve male birds were randomly selected from the control group at 43 d of age. These birds were only given water for 8 h prior to slaughter. Six of the twelve broilers were given control water and the others consumed acidified drinking water (pH 3). In the acidified group, formic acid was added to the control water until the pH was adjusted to 3. At the end of the 8-h period, birds were slaughtered, manually eviscerated and each whole carcass was placed into a sterile bag to create microbial hygiene ambiance. Then the bags in ice were immediately transferred to the laboratory at Bornova Veterinary Control and Research Institute. The microbiological analyses (*Salmonella, total organism* and *E. coli*) were performed in accordance with the procedures recommended by the Turkish Standard Institute (TS, 1979; TS, 1996; TS, 2000).

Chemical analyses

Experimental diets were ground through a 1 mm screen in preparation for chemical analysis. Dry matter, crude protein, ether extract, crude ash, crude fibre, starch, sugar, total calcium and phosphorus were analysed according to Verband Deutscher Landwirtschaftlicher Untersuchungs- und Forschungsanstalten, VDLUFA (Naumann and Bassler, 1993). Estimates of metabolisable energy (ME) were based on protein, ether extract, starch and sugar levels determined on the experimental diets (Rose, 1997).

Data and statistical analysis

Microbial colonies were logarithmically transformed prior to analysis to achieve homogeneity of variance and were expressed as log 10 cfu. The recorded data were analysed by general linear model ANOVA using JMP (SAS Institute, 2000). Results were expressed as means±standard error of the mean (SEM).

RESULTS AND DISCUSSION

Body weight, feed intake, feed conversion ratio and mortality of male broilers given control and acidified water are presented in Table 2. The reduction of water pH from 7.4 to 4.5 with formic acid supplementation significantly decreased body weights at 21 and 42 d of age. However, feed intake, feed conversion ratio and mortality were not negatively affected by acidified drinking water. Our results are in agreement with the observations of Vieira et al. (2005) that acid mixture supplementation at different levels to water led to significantly reduced body weight gain, but did not affect feed conversion ratio and mortality in broilers. However, our results contrast with the findings of Pesti et al. (2004) indicating that acidified drinking water increased body weight in comparison to normal drinking water (2,146 g vs. 2,117 g). In addition, Watkins et al. (2004) and Cornellison et al. (2005) found that water acidification did not affect the performance of turkeys and broilers. The discrepancies in these results are possibly consequences of differences in the type and concentration of organic acid used in the studies.

Overuse of organic acids such as citric and acetic acids might reduce water and feed intake due to the strong taste of water and depress growth performance of broilers. In addition, acidified water might lead to sub-clinical intestinal problems, in spite of the fact that chickens might rapidly adapt to acid water (pH 3, 4, or 5) (Oviedo, 2006). In a study conducted by Daskiran et al. (2004), it was stated that early exposure to dietary acidifier might cause an adaptation to acidifier in birds and reduce the subsequent therapeutic activity of acidifier. Therefore, they proposed to use the acidifiers in the grower phase rather than in the starter phase in order to reduce economic losses from heat stress.

Formic acid administration in the drinking water slightly decreased the gizzard pH value compared to the unsupplemented control group, but the difference was not statistically significant (Table 2). In agreement with our results, several researchers (Watkins et al., 2004; Cornellison et al., 2005) noted that water acidified (pH 4 or 6) by using different acids did not cause significant reduction in the gizzard pH of broilers and turkeys. Watkins

Table 2. The effects of formic acid administration in the drinking water on performance and intestinal microflora

Traits	Experimental groups		Probability
	Control	Formic acid	(p-value)
Body weight (g/bird)			
21 d	752.29±5.59[a]	731.96±6.48[b]	0.0185
42 d	2,257.06±19.92[a]	2,193.30±19.32[b]	0.0224
Feed intake (g/bird)			
0-21 d	781.57±11.53	792.45±14.29	0.5852
22-42 d	2,755.04±72.78	2,706.63±35.83	0.5828
0-42 d	3,536.61±82.99	3,499.08±32.04	0.6948
Feed conversion ratio (g/g)			
0-21 d	1.12±0.02	1.15±0.01	0.2756
22-42 d	1.89±0.07	1.91±0.01	0.7830
0-42 d	1.64±0.05	1.67±0.00	0.6720
Mortality (%)			
0-42 d	5.19±1.79	3.18±1.41	0.3775
Gizzard pH	3.67±0.09	3.52±0.16	0.4359
Intestinal microflora			
Total organisms (log_{10} cfu/g)	6.17±0.62	5.84±0.50	0.7385
E. coli (log_{10} cfu/g)	4.15±0.96	4.02±0.78	0.9355
Salmonella-positive intestinal content (positive samples/total samples)	2/6	1/6	0.8858

[a-b] Means within a row with different superscripts differ significantly (p<0.05).

et al. (2004) also reported that this result might be expected because of the acidification of the proventriculus contents with hydrochloric acid secreted by gastric glands before passing to the gizzard.

The differences in the counts of *total organism* and *E. coli* in the digesta content of intestine were not statistically significant between the treatments. The *total organism* counts for control and acidified groups were 6.17 and 5.84 log cfu/g, respectively. The intestinal *E. coli* population was 4.15 and 4.02 log cfu/g in birds given control and acidified water, respectively. Moreover, the use of formic acid in the drinking water did not significantly affect the number of *Salmonella*-positive intestine (Table 2). Our results showed that acid water treatment did not cause a significant reduction in intestinal pathogens, which contrasts with the observations of Chaveerach et al. (2004). In a previous study, Mohyla et al. (2007) observed that *Salmonella* load was significantly reduced in the upper digestive tract but not in the lower digestive tract when acidified sodium cholorite was added to the drinking water at a level of 600 ppm for the last 24 h or 5 d. Similary, Van Immerseel et al. (2006) reported that acids from feed and water were not effective further down the intestinal tract. According to some authors, most of the short-chain fatty acids (i.e. propionic, formic) used in diets or water are metabolised in the upper gastro-intestinal segments of poultry (Hume et al., 1993; Thompson and Hinton, 1997). Thus, their role in modifying host microflora populations in the lower parts is limited (Józefiak et al., 2010). Recently, some researchers have suggested transport of short-chain fatty acids further down the gastro-intestinal tract by micro-encapsulation in a lipid shell. The protective lipid matrix used for micro-encapsulation allows organic acids to have an effect all along the gastro-intestinal tract, since they are slowly released during digestion (Fernández-Rubio et al., 2009; Van Immersell et al., 2009). Gheisari et al. (2007) found that supplementation of 0.2% protected organic acids to the diet might improve the proliferation of useful microflora and diminish the population of harmful bacteria in poultry gut contents. Other workers have reported that medium-chain fatty acids (C6 to C12; caproic, caprylic, capric and lauric acids) appear to be much more effective against *Salmonella* than short-chain fatty acids (C≤4; formic, acetic, propionic and butyric acids) (Van Immerseel et al., 2006). Therefore, Del Alamo et al. (2007) recommended inclusion of a mixture of short- and medium-chain fatty acids in the diet of broilers affected by malabsorption syndrome. Moreover, many microorganisms possess an adaptive stress response that gives them survival ability when exposed to extremely acidic environments. This acid-tolerance response has been studied in *E. coli* and *Salmonella enterica* and demonstrated that exposure to sublethal pH induces the expression of numerous acid-shock proteins which promote bacterial survival in subsequent extreme

Table 3. The effects of formic acid administration in the drinking water during the pre-slaughter period on carcass contamination

Traits	Experimental groups		Probability
	Control	Formic acid	(p-value)
Total organism ($\log_{10}$ cfu/g)	4.44±0.33	3.96±0.41	0.3880
E. coli ($\log_{10}$ cfu/g)	2.85±0.28	2.20±0.35	0.1863
Salmonella-positive carcass (positive samples/total samples)	0/6	0/6	

acid environments (Moat et al., 2002). In the present study, using acidified drinking water from early in life might have enabled an adaptation of microorganisms to acidic conditions.

The administration of formic acid to the drinking water for 8 h before slaughter did not significantly decrease *total organism* and *E. coli* counts of the carcass. Besides, no *Salmonella* were detected in carcass samples of any of the treatment groups (Table 3). According to De Avila et al. (2003), the addition of organic acids to the drinking water, if started 24 h before the beginning of pre-slaughter feed withdrawal, might help to reduce crop contamination by *Salmonella enteritidis*. As shown in Table 3, formic acid application during the pre-slaughter fasting period did not reduce carcass contamination, which might be the result of a more hygienic evisceration process and/or lower microbial load in the gastro-intestinal tract.

Based on the results of the present study, it can be noted that formic acid administration in the drinking water does not have beneficial effects on performance, microbiological profile of the intestine and carcass contamination in broilers under high ambient temperature. The antimicrobial and growth-promoting effects of organic acids are related to their form (protected or unprotected), type and concentration. The use of the right combination of organic acids would be more effective in view of the synergistic activity between them.

Currently, the demand for organic animal products which do not contain antibiotics and synthetic chemicals is increasing. However, the contamination risks of organic products might be high by virtue of not using antibiotics and animals being in contact with the environment. Consequently, further research on the addition of organic acids to the diet, water or litter in organic poultry production would be beneficial.

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