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Effects of Dietary Acetic Acid on Intestinal Microbiota, Serum Components, Internal Organs and Performance of Broilers

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ABSTRACT

An experiment has been conducted to evaluate the impacts of acetic acid on gut microflora, blood chemicals, and productive traits of broiler chickens. A total 270 day-old Ross 308 chicks were randomly divided into 6 treatments. Each treatment consists of 45 birds and three replicates of 15 birds per replicate. From day one, birds were fed either a corn-soybean meal basal diet supplemented with different levels of diluted acetic acid (5%) (0, 1, 2, 4, 8, and 10 percent of as fed basis diet). Four birds from each pen (12 per treatment) were randomly selected for slaughter and collection of blood samples and microbial study at day 42. There were no significant effects of experimental treatments on feed intake, body weight gain, feed conversion ratio (FCR), and mortality of birds (P>0.05). Relative weight of internal organs was not affected by dietary acetic acid. Acetic acid at the level of 8% significantly increased the blood urea, but other serum components did not influenced by addition of acetic acid to the ration. Unexpectedly, total counts of bacteria at 8 and 10 percent of acetic acid have been sharply increased which might be due to resistance of some bacteria to overuse of this organic acid.

Keywords: organic acid, Ross 308, weight gain, blood constituents.

INTRODUCTION

Bacterial contamination of poultry products continues to be a major concern for consumers. 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 (Luckstadt, 2007) which might have some other beneficial impacts such as on bone tissues (Khodambashi Emami *et al.*, 2013; Mohammadpour *et al.*, 2014). 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 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 a preservative and acidifier, in order to replace antibiotics as growth promoters and prevent or control pathogens (Papatsiros *et al.*, 2012; Sugiharto, 2014).

Organic acids are any organic carboxylic acid of the general structure R-COOH. The organic acids have a long history of use as preservatives and functional feed additives (Dibner and Buttin, 2002). Moreover, Saki *et al.*, (2012) demonstrated that organic acids significantly reduced *Enterobacteriaceae* counts in ileum and caecum of broiler chickens at 21 and 42 days of age. Various organic acids which are particularly effective against acid-intolerant species such as *E. coli*, *Salmonella* and *Campylobacter* have been used in feed by different researchers (Izat *et al.*, 1990; 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). The objective of this study was to evaluate the effects of dietary acetic acid as an organic acid on the growth performance, intestine microbial population and blood serum components in broiler chicken from hatch to 42 d.

MATERIALS AND METHODS

Birds, Diets and Experimental Design

A total number of 270 day-old straight chicks (Ross 308) were purchased form a local hatchery. All the chicks were weighed individually (Mean = 40 g) and randomly divided into 6 treatments. Each treatment consists of 45 birds and three replicates of 15 birds per replicate. The initial house temperature was set at 32 °C and gradually decreased to reach 24 °C at 28 d. A lighting schedule of 24 h illumination with approximately 20 lx was used for the entire period. From day one, birds were fed either a corn-soybean meal basal diet (control with no additive) or the basal diet supplemented with diluted acetic acid (5%) (1%, 2%, 4%, 8% and 10%). The basal diet (Table 1) were formulated to meet the nutrient requirements of the broiler chickens as recommended by Ross 308 broiler management guide (Aviagen, 2009). Feed and water were offered *ad libitum* throughout the trial.

Growth Performance

Body weight gain per pen was evaluated at 42 d. Feed intake and feed conversion ratio were determined and calculated during each phase of whole period. Mortality was recorded daily by counting the number of dead birds.

Blood Sampling and Determination of Serum Components

At the end of the experiment (42 d), 4 birds from each pen (12 per treatment) were randomly selected and blood samples were collected from the wing vein with a 25G needle. Serum was obtained by centrifugation of the coagulated blood (3000 rpm for 10 min). Glucose, Cholesterol, Triglycerides, HDL, LDL, Urea, Creatinine, Aspartate Amino Transferase (AST), Alanine Amino Transferase (ALT) and Alkaline Phosphatase (ALP) were analyzed by an automatic analyzer (Clima, Ral. Co, Espain).

Internal Organs

At the end of the experiment, the weights of liver, pancreas and spleen were measured. Relative organ weights were calculated as [organ weight (g)/live body weight (g)] ×100. Small intestine of birds was opened immediately after killing and relative length [length of intestine to live body weight as percentage (cm/g)] was measured.

Microbial Population

Microbial enumeration was determined by serial dilution (10^{-4} to 10^{-7}) of fecal samples before inoculation onto sterile agar. For total aerobic enumeration samples were cultured from serial dilutions on brain heart infusion agar (BHI). In addition, *E. coli* and *Lactobacilli* were grown on eosin methylene blue agar (EMB) and MRS agar, respectively. Plates for *E. coli* and aerobic bacteria were incubated aerobically at 37°C. Plates for *Lactobacillus* were incubated anaerobically at 37°C. Colony forming units (CFU) were defined as being distinct colonies measuring at least 1 mm in diameter (Alzawqari *et al*., 2013a). The effects of different levels of acetic acid on gut microbial population of broiler chickens are shown in table 4.

Statistical Analysis

Primary data of microflora number 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 tests was used to compare the means. All statements of significance were based on probability of $P<0.05$.

Table 1: The composition of basal diets

Item	Starter	Grower	Finisher
Ingredients (%)			
Corn	55.4	59.2	64.5
Soybean meal	39	34	28
Vegetable oil	1.2	3	3.7
Oyster shell	1.1	1.1	1.05
Dicalcium phosphate	2	1.5	1.55
Common salt	0.3	0.35	0.35
L-Lysine HCl	0.15	0.10	0.10
DL-Methionine	0.25	0.15	0.15
Vitamin E	0.1	0.1	0.1
Vitamin and mineral premix [1]	0.5	0.5	0.5
Calculated contents (%)			
ME (kcal/kg)	2851	3000	3094
Crude protein	21	19.17	17.07
Calcium	0.97	0.93	0.86
Available phosphorus	0.48	0.43	0.35
sodium	0.16	0.17	0.17
Lysine	1.38	1.15	1.01
Methionine	0.70	0.55	0.48
Methionine+Cystine	1.03	0.86	0.78

1vitamin and mineral premix supplied per kilogram of diet: vitamin A, 10000 IU; vitamin D3, 9800 IU; vitamin E, 121 IU; B12, 20 µg; riboflavin, 4.4 mg; calcium pantothenate, 40 mg; niacin, 22 mg; choline, 840 mg; biotin, 30 µg; thiamin, 4 mg; zinc sulfate, 60 mg; manganese oxide, 60 mg.

Table 2: Effects of different levels of dietary acetic acid on body weight gain, feed intake, and feed conversion ratio (FCR) of broiler chickens at 42 d of age

Treatments (acetic acid %)	Body weight gain (g)	Feed intake (g)	FCR
0	2272	4915	2.16
1	2382	5092	2.13
2	2270	4674	2.07
4	2423	4796	1.98
8	2243	4011	2.05
10	2183	4664	2.13
±SEM	61.5	172.4	0.093
P-value	0.131	0.396	0.766

RESULTS

The results of this trial are presented in Tables 2, 3, 4, 5. As Table 2 indicates, body weight of broilers receiving 4 % acetic acid was slightly higher as compared to other treatments; but this improvement was not significant. Also, The FCR of broilers receiving 4% acetic acid was better than other groups, albeit it was not pronounced. There were no differences in feed intake among experimental treatments (P>0.05). Relative weight or length of internal organs was not affected by dietary acetic acid (Table 3). The mean values of serum components in broiler chicken fed acetic acid supplemented diets are shown in Table 4. Acetic acid at the level of 8% significantly increased the blood urea. Also, total counts of bacteria at 8 and 10 percent of acetic acid have been sharply raised. However, the other characteristics did not impressed by treatments (Tables 4 and 5). Mortality remained below 5% in all groups without any differences between the groups.

DISCUSSION

Performance and Internal Organs

Supplementing organic acid in this study did not have significant effect on body weight gain, but positive effects of dietary acetic acid on feed efficiency in certain dose (4%) were slightly observed herein. On the other hand, according to the Table 2, the best result for performance criteria was in group with 4% acetic acid. Positive effects of organic acid supplementations on growth performance of chickens were notified in previous studies (Esmaeilipour *et al.*, 2011; Garcia *et al.*, 2007; Islam, 2012). For instance, improvement of feed intake and FCR due to addition of 0.5% citric acid in broilers diet was reported by Chowdhury *et al.* (2009).

Nevertheless, some researchers reported no influence of organic acids on body weight and FCR of broilers (Isabel and Santos, 2009). Boling *et al.*, (2000) mentioned that citric acid did not improve performance of laying hens fed a corn-soybean meal diet containing sufficient calcium. Also, calcium propionate and calcium formate did not influence weight gain, but deteriorated FCR (Isabel and Santos, 2009).

Table 3: Effects of different levels of dietary acetic acid on liver, pancreas, spleen, and small intestine of broiler chickens at 42 d of age

Treatments (acetic acid %)	Liver[1]	Pancreas[1]	Spleen[1]	Small intestine[2]
0	2.31	0.22	0.10	9.31
1	2.13	0.24	0.07	9.06
2	2.32	0.23	0.10	8.82
4	2.23	0.24	0.10	9.52
8	2.32	0.25	0.11	9.57
10	2.26	0.21	0.09	8.39
±SEM	0.106	0.014	0.011	0.335
P-value	0.771	0.443	0.196	0.141

[1] Relative weight of organ to live body weight as percentage (g/g)
[2] Relative length of intestine to live body weight as percentage (cm/g)

Table 4: Effects of different levels of dietary acetic acid on serum Components of broiler chickens at 42 d of age

Treatments (acetic acid %)	Glucose	Cholesterol	Triglycerides	HDL	LDL	Urea	Creatinine	AST*	ALT*	ALP*
0	197	130	69	59	94	0.16[b]	0.42	331	4	608
1	195	122	66	56	89	0.50[b]	0.43	336	5	386
2	202	129	79	59	83	0.00[b]	0.45	315	4	1079
4	178	118	83	54	86	0.00[b]	0.43	364	9	598
8	202	137	77	62	98	2.00[a]	0.43	356	4	394
10	193	115	70	54	86	0.50[b]	0.41	416	5	420
±SEM	8.45	8.72	7.96	4.14	6.25	0.461	0.023	57.9	1.2	278
P-value	0.313	0.435	0.533	0.641	0.201	0.038	0.824	0.824	0.056	0.481

a, b Means with different superscripts in each column are significantly different ($P<0.05$).
*AST: aspartate aminotransferase; ALT: alanine aminotransferase; ALP: alkaline phosphatase

Table 5: Effects of different levels of dietary acetic acid on gut microbial population (Log 10 cfu) of broiler chickens at 42 d of age

Treatments (acetic acid %)	Total bacteria	*Lactobacillus*	*E. coli*
0	7.93[ab]	7.11	6.00
1	7.72[b]	7.03	5.74
2	8.00[ab]	6.57	5.98
4	8.07[ab]	7.07	6.54
8	8.49[a]	6.68	6.83
10	8.51[a]	7.21	6.23
±SEM	0.207	0.232	0.366
P-value	0.048	0.216	0.180

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

The addition of citric acid reduced the weight gain but did not modify the feed intake and FCR (Centeno *et al.*, 2007). The inclusion of 20 g/kg of citric acid decreased feed intake, but it did not affect body weight gain of broilers at 24 d of age (Esmaeilipour *et al.*, 2012). Also, in another study, citric acid decreased feed intake and weight gain of broilers (Ao *et al.*, 2009). Pirgozliev *et al.*, (2008) indicated that fumaric acid reduced feed intake of broilers. Islam *et al.* (2008) have conducted a research with fumaric acid from 0 to 7.5 percent of diet in broilers. They concluded that safety margin is about 3%; and 1.25% of this organic acid in the diet was the optimally effective concentration. Furthermore, Runho *et al.*, (1997) observed that fumaric acid improved significantly FCR in broilers fed diets from 0.25 to 1%, because a reduction of consumption was noticed, but growth was unaffected. However, growth promoting effects of organic acids in poultry do not seem to be as remarkable as in pigs (Langhout, 2000).

In our trial, dietary treatments had no significant effect on the relative length of small intestine or proportional weight of liver and pancreas. The results of this experiment are in agreement with other report Esmaeilipour *et al.* (2012) who did not observe any beneficial effect of citric acid on small intestine. Other experiments indicated that the addition of organic acids to diet had no effect on the relative weight of liver (Adil *et al.*, 2010; Antongiovanni *et al.*, 2007; Brenes *et al.*, 2003), and spleen (Çakir *et al.*, 2008). The carcase, breast, and right thigh yields of broilers at 49 d of age were unaffected by formic acid (Garcia *et al.*, 2007). Calcium propionate and calcium formate did not influence carcase weight of broilers (Isabel and Santos, 2009). The relative weight of heart, liver and spleen in broilers was not affected by fumaric acid (Islam *et al.*, 2008). The relative weights of gizzard, liver, spleen, bursa of Fabricius, breast, and abdominal fat were unaffected by phenyllactic acid in broilers (Wang *et al.*, 2010) or other organic acids did not influence breast, thigh, and carcase of quails (Ghosh *et al.*, 2010; Sacakli *et al.*, 2006). However, some workers demonstrated the positive impacts of organic acids on carcase and internal organs of poultry. For instance, Leeson *et al.* (2005) observed that carcass weight and breast meat yield increased in birds fed 0.2% butyric acid.

Also, individual supplementation of ortho-phosphoric, formic and propionic acid and calcium propionate and their blend significantly improved the feed efficiency, proportional weight of hot and dressed carcase and wholesale cuts compared to the control group of birds (Samanta *et al.*, 2008). Broilers consuming acidifier during grower phase exhibited increased carcase, leg quarters, and breast yield (Daskiran *et al.*, 2004). Mohammadpour *et al.*, (2014) indicated that acidifiers in suboptimal diets containing wheat without exogenous enzyme had some beneficial effects on bone mineralization. Also, the Japanese quails fed diet with 1.2% malic acid had a higher gut length compared to the control group, but relative weight of full gut, edible internal organs, and dressing percentage were not altered by organic acid (Ocak *et al.*, 2009). All in all, these organic acids have some advantages on bird performance and internal organs, but there is not consistency among various results due to different circumstances.

Serum Components and Gut Microbiology

Supplementation of organic acids showed no significant (P>0.05) difference in the concentration of serum glucose, cholesterol, triglycerides, creatinine, HDL and LDL among all the groups. These results are in line with previous studies (Adil *et al.*, 2010; Hernández *et al.*, 2006). Furthermore, formic acid addition to broilers diet did not alter the biochemical profile of blood under conditions of good hygiene (Hernández *et al.*, 2006). Also, there was no significant (P <0.05) difference in the ALT, AST and ALP levels between the chicks fed diets supplemented with organic acid and the control group confirming the earlier finding (Abdel-Fattah *et al.*, 2008) which concluded that dietary supplementation of organic acids could be done up to the level of 3% in the diet of broiler chicken without causing any adverse effect on the kidney and liver functions.

In this study, administration of organic acid in low doses (1 and 2%) reduced population of *E. coli*, but in high doses increased the population of these bacteria. Total counts of bacteria at 8 and 10 percent of acetic acid have been sharply increased which might be due to resistance of some bacteria to overuse of this organic acid. In general, diminution of gastrointestinal pH has adverse impacts for colonization of acid-intolerant bacteria such as *Coliforms*. 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 or acts by suppressing the cellular enzyme and transport systems (Luckstadt, 2007; Ricke, 2003; Strauss and Hayler, 2001). Acids with a high pK_a (which are 50 percent dissociated), longer-chain-length acids and unsaturated acids are the most effective.

There are several perpers indicating fruitful use of organic acids as antibiotic replacement in poultry. For example Alzawqari *et al.* (2013a, b) showed that the addition of acetic or citric acids to drinking water resulted in significant reduction of *clostridiums* and *coliforms* in gizzard, cecal and fecal contents of birds with acidified water before slaughter in comparison to the control and feed withdrawal treatments. Also, Philipsen (2006) that revealed 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% malic acid to drinking water significantly reduced *E. coli* counts in the small intestine of layer chickens. Chaveerach *et al.* (2002) indicated that the efficacy of organic acids in controlling microbes such as *Campylobacter* is influenced by concentration, form of the acid, and the degree of any dissociation. The results of the use of the mixtures of organic acids (formic, acetic, propionic, and hydrochloric acids) demonstrated that although the amounts of accumulated undissociated acids of each acid in combinations are less than in individual use of acids at the same low pH levels, particular at pH 4.5, the mixtures gave a higher reduction rate. They strongly demonstrated that the combination of organic acids gives a remarkably high bactericidal effect on *Campylobacter jejuni/coli* viability in low pH aqueous solutions. The reason for this synergistic effect is, as yet, unknown.

The total counts for control and acidified groups were 7.93 log cfu/g, However, this value was lower in the other groups. This finding is similar to result of previous studies. Aciokgoz *et al.* (2011) reported that formic acid did not significantly change the microflora of broilers. Also, Van Immerseel *et al.*, (2006) reported that acids from feed and water were not effective further down the intestinal tract. It is mentioned that 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 (Thompson and Hinton, 1997). Thus, their role in modifying host microflora populations in the lower parts is limited (Józefiak *et al.*, 2006).

The benefits of using organic acids in animal nutrition, specifically poultry nutrition, could be beyond their antimicrobial activities and modification of gut microbiota. It has been reported that individual organic acid and blends of organic acids, apart from their antimicrobial activity, reduce the digesta pH, improve in digestive enzymes and microbial phytase activity, increase the pancreatic secretion, have beneficial effects on the gastrointestinal microbiota and morphology, stimulate gastrointestinal cell proliferation and have trophic effects on the gastrointestinal mucosa (Dibner and Buttin, 2002).

CONCLUSION

These variations in results may be due to differences in the type of organic acid, dose of organic acid and its use in the diet. For instance, acetic acid at the level of 8% significantly increased the blood urea, but other serum components did not influenced by addition of acetic acid to the ration. Unexpectedly, total counts of bacteria at 8 and 10 percent of acetic acid have been sharply increased which might be due to resistance of some bacteria to overuse of this organic acid.

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