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Effects of Acidifier Supplementation and Toxin Binder on Performance, Carcass, Blood Metabolites, Intestinal Morphology, and Microbial Population in Broiler Chickens

Research Article

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ABSTRACT

An experiment was conducted to investigate the effects of acidifier (in drinking water) and toxin binder on the growth performance, carcass characteristics, blood metabolites, microbial population and intestinal morphology of broiler chickens. A total of 256 one-day old broiler chickens were distributed in four treatments with four replicates per each. The experimental treatments were: 1) control group (without acidifier and toxin binder), 2) group supplemented with acidifier, 3) group supplemented with toxin binder and 4) group supplemented with both acidifier and toxin binder. At 24 and 42 days of age, four birds per each treatment were selected and carcass traits, blood metabolites, microbial population and intestinal morphology were determined. The results showed that the experimental treatments had no significant effect on the growth performance of broiler chickens. The liver and heart weight was higher in broilers that received toxin binder ($P<0.05$). The experimental treatments did not alter the immune response and microbial population of the broiler chickens. The results indicated that the lowest serum concentration of cholesterol and triglyceride was observed in broiler chickens received treatment acidifier in combination with toxin binder ($P<0.05$). However, the birds fed toxin binder had a lower serum concentration of total protein, uric acid and liver enzyme activity ($P<0.05$). In the intestinal morphology, use of acidifier in combination with toxin binder improved the intestinal morphological traits in broiler chickens ($P<0.05$). On the basis of the present results, it can be used acidifier in combination with toxin binder in broiler chickens without any negative effect on the growth performance.

KEY WORDS acidifier, broiler, intestinal morphology, microflora, toxin binder.

INTRODUCTION

Organic acids (acidifiers) are an important group of antibiotic alternative compounds and their use in poultry diets has increased in recent decades. It is well known that these supplements have a great impact on the function of the gastrointestinal tract of the poultry by changing the microbial population via depolarization of the bacterial membrane, a change in its internal acidity (Cherrington *et al.* 1990) and making better use of the nutrients by the birds. In recent years, the utilization of organic acids in drinking water compared to feed has increased. On the other hand, it is reported that drinking water is one of the most important factors to spread of microorganism infections on the poultry farm (Eftekhari *et al.* 2017). Therefore, it seems that the addition of the organic acids to drinking water may have good influence on the increasing of the useful bacteria in the digestive tract of the broilers. In this regard, several

studies showed the positive impacts of organic acids on the growth performance (Ogunwole *et al.* 2011), nutrient digestibility (Ao *et al.* 2009b), intestinal morphology (Eftekhari *et al.* 2015) and microbial population (Chaveerach *et al.* 2004) of broiler chickens. However, there is limited information regarding to the effect of other feed supplements such as mycotoxin binders on the efficacy of organic acids in broiler nutrition.

The contamination of the feed ingredients with mycotoxins is one of the most important problem associated with feeding of broiler chickens (Manafi and Khosravinia, 2012). Mycotoxins are toxic compounds of fungi that grow on poultry feed and cause undesirable effects in broiler growth performance. In recent years, addition of toxin binders to poultry feed is widely increased to overcome these undesirable effects. Several reports have shown that fungal toxin adsorbents are able to reduce the amount of poison absorption from the gastrointestinal tract and increase nutrient utilization and finally increase the growth performance of broiler chickens (Pappas *et al.* 2014; Agboola *et al.* 2015).

It is hypothesized that acidity changes in the intestinal tract due to the addition of the organic acids to drinking water may affect the efficiency of other additives such as toxin binders in broiler chickens. Therefore, the objective of this research was to investigate the effects of acidifier supplementation and toxin binder in broiler chicken diets on growth performance, carcass characteristics, intestinal morphology, and microbial population.

MATERIALS AND METHODS

All procedures used in this experiment were approved by the Department Science and Research Branch, Islamic Azad University, Tehran, Iran.

Two hundred and fifty five1-d-old broiler chickens (Ross 308) were obtained from a commercial hatchery (Zarbal Company, Amol, Iran) and randomly allocated into 4 treatments with 4 replicate pens of 16 broiler chickens per pen. The broiler chickens were raised in floor pens (1.0×1.7 m) for the experimental period of 42 d. Each pen was equipped with a separate feeder and a manual drinker. The house temperature was maintained at 35 ± 1 ˚C during the first week, and it was reduced 2 ˚C per week until reaching the temperature of 23 ˚C. The broiler chickens were provided access to feed and water *ad libitum*.

The experiment used a completely randomized design with four treatments, including a control group, control group with dietary toxin binder, control group with acidified drinking water and control group with both of them. The commercial toxin binder used in the present experiment was NufotoxPlus (Spain) and added 2 g/kg to the diet, as recommended by the supplier. The drinking water was supplied with 1 mL/L of NufocidL as an organic acid supplement. The experimental diets were formulated to meet or exceed the energy and nutrient requirements (Aviagen, 2009). The composition of the experimental diets is presented in Table 1.

Growth performance and carcass characteristics

The broiler chickens were fed the 4 experimental diets until 42 d of age. Feed intake and body weight gain of each pen was measured at the end of each phase. Feed conversion ratio for each pen was calculated by dividing feed intake by body weight gain. Mortality was recorded and weight gain and feed consumption data were corrected accordingly.

At 21 and 42 days of age, four broiler chickens per each treatment, which was close to the mean weight of the pen, was selected and killed by cervical dislocation for the assessment of carcass characteristics, intestinal morphology, and microflora population. After removing the viscera manually, carcass characteristics, including the weight of the breast, thigh, liver (without gallbladder), pancreas, heart, gizzard, proventriculus, crop and abdominal fat was recorded. The carcass data are presented based on percent of live weight of each broiler chicken.

Jejunum morphology

After removing the intestinal contents, 3 cm lengths of the duodenum, jejunum (mid- point of jejunum) and ileum (5 cm after Meckel's diverticulum) were removed for morphological measurements. The segments were flushed clean with phosphate buffered saline to avoid damage to the tissues. Then, all samples were fixed in formaldehyde solution for 1 h. Samples were then transferred in 50% ethanol solution. A 0.5 µm thickness section was processed, embedded in paraffin, stained with eosin blue, and examined with a light microscope. The villus height and crypt depth were measured with linear scaled graticule. The number of goblet cells was measured by 25 squared graticule. Ten microscopic fields per bird were measured, and the average value was expressed as the morphological value for each broiler chicken (Eftekhari *et al.* 2015).

Microbial enumeration

At 21 and 42 days of age, 4 broiler chickens per treatment were selected, weighed, and killed by cervical dislocation. The intestinal tract of each broiler chicken was removed and samples of fresh digesta (1 to 2 g) from the ileum (Meckel's diverticulum to 1 cm proximal to the ileocecal junction) were collected and mixed both samples together and gently placed in sterile sampling tubes. Samples were subjected on ice until they were transported to the laboratory for enumeration of microbial populations.

Table 1 Composition of basal diets (as-fed basis)

Ingredient (% unless stated otherwise)	Starter	Grower	Finisher
	d 0 to 10	d 11 to 24	d 25 to 42
Corn grain	55.86	60.76	66.34
Corn gluten meal	3.00	0	0
Soybean meal (440 g CP/kg)	35.84	33.74	27.82
Soybean oil	0.85	1.35	1.89
Sodium bicarbonate	0.24	0.19	0.30
Oyster shell	0.82	0.74	0.70
Dicalcium phosphate	2.20	1.95	1.69
Common salt	0.19	0.22	0.17
Vitamin premix[1]	0.25	0.25	0.25
Mineral premix[2]	0.25	0.25	0.25
DL-Met	0.25	0.23	0.20
L-Lys HCl	0.21	0.10	0.11
L-Thr	0.04	0.02	0.03
Choline chloride	0	0	0.05
Chemical composition (calculated)			
Metabolizable energy (ME) (kcal/kg)	2850	2900	3000
Crude protein (CP)	22.52	20.08	17.82
Ca	0.93	0.84	0.75
Available P	0.47	0.42	0.37
Lys	1.20	1.54	1.40
Met + Cys	0.958	0.856	0.771
Thr	0.886	0.788	0.707
Arg	1.440	1.334	1.159
Met	0.593	0.528	0.474

[1] Provides per kilogram of diet: vitamin A: 9000 IU; vitamin D_3: 2000 IU; vitamin E: 18 IU; Menadion: 2 mg; Thiamine: 1.8 mg; Riboflavin: 6.6 mg; Niacin: 30 mg; Pyridoxine: 3 mg; vitamin B_{12}: 15 µg; D-pantothenic acid: 100 mg; Folic acid: 1 mg; Biotin: 0.1 mg; Choline chloride: 500 mg and Antioxidant: 100 mg.
[2] Provides per kilogram of diet: Mn: 100 mg; Zn: 84.7 mg; Fe: 50 mg; Cu: 10 mg; I: 1 mg and Se: 0.2 mg.

One g per each sample was serially diluted from 10^{-1} to 10^{-7} in sterilized physiological saline solution (NaCl 85%). Then 0.1 mL of each diluted sample was plated onto the following media. *Escherichia coli* was cultured on eosin methylene blue agar (Merck, Darmstadt, Germany) at 37 ˚C for 24 h. *Lactobacilli* bacteria were enumerated on de Man, Rogosa, Sharpe agar (Merck, Darmstadt, Germany) after incubation for 48 to 72 h at 37 ˚C. The population of *Escherichia coli* and *Lactobacilli* bacteria was calculated as the log 10 of colony forming units (cfu) per gram of digesta.

Blood metabolites and antibody titer

At 21 and 42 days of age, in order to determine the serum biochemical metabolites, blood samples were collected from the wing vein of each bird. Then, blood samples were centrifuged at 5000 × g during 5 min at 23 ˚C. The serum samples were used to measure serum concentrations of glucose, cholesterol, triglyceride, high-density lipoprotein (HDL), total protein, albumin, uric acid, aspartat aminotransferase (AST) and alanine aminotransferase (ALT) by spectrophotometer (Shimadzu, Japan) using Pars azmoon kits (Pars Azmoon Company, Iran). Besides, the antibody titer against Infectious bronchitis (IBD) and Newcastle (ND) diseases was measured at 17 and 28 days of age, respectively.

Statistical analysis

Statistical analysis was conducted using the general linear model procedure of SAS (SAS, 1999) to evaluate the effects of treatments on growth performance, carcass traits ,intestinal morphology, microflora and some blood parameters of broiler chickens. The treatment means with significant differences were compared using Duncan multiple range test. Differences among means were considered statistically significant at $P < 0.05$.

RESULTS AND DISCUSSION

The effect of experimental treatments on the growth performance and carcass characteristics of broiler chickens was presented in Tables 2 and 3. None of the growth performance traits including body weight gain, feed intake and feed conversion ratio were significantly affected by the experimental treatments. Similarly, carcass characteristics of the broiler chickens were not significantly influenced by the experimental treatments.

Tables 4 and 5 present the blood biochemical parameters of broiler chickens at 24 and 42 days of age, respectively. According to the results of the Table 4, the broiler chickens that received acidifier in combination with toxin binder had the highest serum level of total protein at 24 day of age ($P<0.05$).

Table 2 Effects of treatments on weight gain, feed intake and feed conversion ratio in broiler chickens[1]

Age	Treatments				SEM	P-value
	Control	Acidifier (AF)	Toxin binder (TB)	AF + TB		
	Weight gain (g/bird/d)					
d 1 to 10	197.28	201.34	201.00	194.69	5.88	0.83
d 11 to 24	591.50	616.30	601.97	591.48	12.78	0.49
d 25 to 42	1313.9	1321.1	1296.0	1360.1	30.51	0.53
d 1 to 42	2102.7	2138.8	2098.9	2146.2	27.15	0.51
	Feed intake (g/bird/d)					
d 1 to 10	262.81	263.11	263.91	262.56	1.47	0.92
d 11 to 24	965.16	982.84	988.23	972.33	12.60	0.58
d 25 to 42	2805.0	2895.1	2825.0	2838.8	42.11	0.50
d 1 to 42	4032.9	4140.8	4077.1	4073.6	48.04	0.48
	Feed conversion ratio					
d 1 to 10	1.33	1.30	1.31	1.32	0.035	0.83
d 11 to 24	1.63	1.59	1.64	1.64	0.030	0.72
d 25 to 42	2.14	2.19	2.19	2.08	0.065	0.66
d 1 to 42	1.92	1.94	1.94	1.90	0.035	0.80

[1] Data represent the mean of 4 replicate pens of 16 broiler chickens per pen.
SEM: standard error of the means.

Table 3 Effects of treatments on carcass characteristics (g/100 g body weight) and intestine length (cm) in broiler chickens at 42 days of age[1]

Parameters	Treatments				SEM	P-value
	Control	Acidifier (AF)	Toxin binder (TB)	AF + TB		
Breast	35.51	36.81	36.23	37.56	1.16	0.76
Thigh	30.89	31.57	32.48	32.59	0.90	0.51
Liver	2.78	2.61	2.94	2.92	0.16	0.45
Heart	0.52	0.42	0.59	0.51	0.04	0.16
Gizzard	2.13	1.80	1.75	1.78	0.26	0.71

[1] Data represent the mean of 4 broiler chickens per treatment.
SEM: standard error of the means.

Table 4 Effects of treatments on microbial counts (at 24 and 42 days of age) and immune response in broiler[1]

Parameters	Treatments				SEM	P-value
	Control	Acidifier (AF)	Toxin binder (TB)	AF + TB		
24 day of age						
E. coli (log_{10} cfu/g)	8.05	7.17	7.77	7.43	0.38	0.42
Lactobacillus (log_{10} cfu/g)	7.14	7.47	7.79	7.52	0.34	0.61
42 day of age						
E. coli (log_{10} cfu/g)	8.47	8.32	8.26	8.27	0.12	0.59
Lactobacillus (log_{10} cfu/g)	7.53	7.83	7.58	7.56	0.31	0.89
Immunity (log 2)						
Anti-Newcastle disease virus (NDV) titer	3.63	3.55	3.75	3.75	0.18	0.84
Anti-Infectious bronchitis (IBD) titer	223.0	240.7	236.2	247.2	9.23	0.10
Lymphoid organs (%)						
Bursa	0.21	0.20	0.19	0.17	0.03	0.84
Spleen	0.14	0.17	0.16	0.16	0.02	0.88

[1] Data represent the mean of 4 broiler chickens per treatment.
SEM: standard error of the means.

On the other hand, the serum concentration of cholesterol, triglyceride, total protein, uric acid and AST (liver enzyme) were affected by the experimental treatments at 42 day of age. The inclusion of dietary toxin binder in combination with acidifier decreased the serum level of cholesterol and triglycerides ($P<0.05$).

Toxin binder supplementation declined the concentration of total protein in the serum of the broiler chickens ($P<0.05$). In comparison with the control group, the birds received toxin binder and acidifier alone or in combination had lower serum concentration of uric acid and AST ($P<0.05$).

Table 5 Effects of treatments on the serum metabolites in broiler chickens at 24 days of age[1]

Parameters	Treatments				SEM	P-value
	Control	Acidifier (AF)	Toxin binder (TB)	AF + TB		
Glucose	234.25	224.00	206.01	185.02	12.20	0.07
Cholesterol	128.71	150.82	136.06	114.55	9.90	0.12
Triglyceride	87.80	90.98	100.49	71.22	9.10	0.23
HDL	78.25	76.00	71.50	79.75	2.90	0.25
Total protein	3.59[b]	3.52[b]	3.46[b]	3.99[a]	0.09	0.006
Albumin	1.72	1.86	1.75	1.95	0.08	0.35
Uric acid	6.94	6.68	6.75	7.14	0.14	0.17
AST	207.0	199.1	228.2	230.7	13.95	0.32
ALT	2.88	2.24	2.81	3.24	0.26	0.11

[1] Data represent the mean of 4 broiler chickens per treatment.
HDL: high-density lipoprotein; AST: aspartat aminotransferase and ALT: alanine aminotransferas.
The means within the same row with at least one common letter, do not have significant difference (P>0.05).
SEM: standard error of the means.

The experimental treatments had no significant effect on the antibody titer against Newcastle and Infectious Bronchitis virus diseases in broiler chickens (Table 5). On the other hand, the relative weight of bursa and spleen as lymphoid organs wan not affected by the experimental treatments in broiler chickens.

The results of Table 6 indicated that the experimental treatments had no significant effect on the intestinal microbial population including *Lactobacilli* and *E. coli* in broiler chickens at 24 and 42 days of age. The results of the intestinal morphology of broiler chickens at 24 and 42 day of age are presented in Table 7. The results showed a significant effect for duodenal villus length and jejunal villus width at 24 day of age. The duodenal villus length was greater in broilers that received toxin binder in combination with acidifier (P<0.05), while the birds fed control diet had the highest value of jejunal villus width (P<0.05). At 42 day of age, the duodenal crypt depth was higher in the birds received toxin binder and acidifier alone or in combination compared with the control group (P<0.05). The results also indicated that the use of toxin binder alone or in combination with acidifier increased the jejunal crypt depth compared with the control group (P<0.05). According to the results of Table 7, the ileal villus length and ileal crypt depth were greater in broilers that received toxin binder in combination with acidifier (P<0.05).

In the present experiment, the utilization of toxin binder and acidifier alone or in combination did not affect the growth performance and carcass traits of broiler chickens. These results did not support the previous findings (Emami *et al.* 2013; Mohamed *et al.* 2014; Agboola *et al.* 2015).

Agboola *et al.* (2015) reported that the use of an absorbent of fungal toxins compared to antibiotics growth promoters improved the body weight gain in broiler chicks. Also, in contrast to our findings, Wang *et al.* (2006) observed an increase in the growth performance of broiler chickens that received an absorbent of mycotoxin in corn-infected diets.

In general, the failure to conclude of the previous experiment can be explained by the absence of a challenge associated with antifungal activity in the diet of broiler chickens. It is well documented that the optimal performance of the antifungal compounds in broiler chickens has been observed when the birds subjected to a challenge associated with fungal toxin (Oliveira *et al.* 2015; Manafi *et al.* 2016).

On the other hand, the growth performance parameters and carcass traits were not affected by the experimental treatments in the present study. Organic acids are known as useful compounds that can be utilized as an alternative to antibiotics in poultry diets. In contrast with the present results, several studies have focused on the beneficial effects of acidifiers in both diet and drinking water on growth performance (Ogunwole *et al.* 2011) and nutrient digestibility (Ao *et al.* 2009a) in broiler chickens. In recent years, the use of acidifiers in drinking water has shown an increase compared to feed, which can be due to the reduction of feed machine corrosion and the reduction of the effects of these compounds during the process of the pellet production (Eftekhari *et al.* 2015). Also, the use of organic acids in poultry drinking water may lead to the reduction of pathogenic organisms in water and consequently the reduction of gastrointestinal infections in poultry.

In the present experiment, the use of toxin binder in combination with acidifier increased the serum concentration of broiler chickens at the age of 24 days. However, the toxin binder supplementation unexpectedly declined the concentration of total serum protein in broiler chickens at 42 days of age. It is reported that aflatoxins may cause an extensive changes in poultry including effects on internal organs, growth reduction and reduced serum protein (Leeson *et al.* 1995). According to these authors, fungal toxins can prevent tissue protein synthesis. The inclusion of dietary toxin binder in combination with acidifier decreased the serum level of cholesterol, triglyceride, uric acid and AST in broiler chickens.

Table 6 Effects of treatments on the serum metabolites in broiler chickens at 42 days of age[1]

Parameters	Treatments				SEM	P-value
	Control	Acidifier (AF)	Toxin binder (TB)	AF + TB		
Glucose	236.7	225.0	201.7	233.2	12.41	0.23
Cholesterol	145.5^{a}	132.7^{b}	135.7^{b}	111.2^{c}	3.05	0.001
Triglyceride	94.00^{a}	89.50^{a}	88.25^{a}	72.75^{b}	3.83	0.01
HDL	75.75	79.50	82.50	76.50	3.10	0.43
Total protein	3.56^{a}	3.23^{a}	3.11^{b}	3.31^{ab}	0.11	0.04
Albumin	1.36	1.46	1.44	1.70	0.14	0.38
Uric acid	6.74^{a}	6.23^{b}	6.10^{b}	6.18^{b}	0.15	0.04
AST	309.0^{a}	276.5^{b}	264.7^{b}	267.2^{b}	7.00	0.001
ALT	3.29	2.70	2.36	2.71	0.34	0.33

[1] Data represent the mean of 4 broiler chickens per treatment.
HDL: high-density lipoprotein; AST: aspartat aminotransferase and ALT: alanine aminotransferas.
The means within the same row with at least one common letter, do not have significant difference (P>0.05).
SEM: standard error of the means.

Table 7 Effects of treatments on intestinal morphology indices in broiler chickens at 24 days of age[1]

Parameters	Treatments				SEM	P-value
	Control	Acidifier (AF)	Toxin binder (TB)	AF + TB		
Duodenum						
Villus length (µm)	1308^{b}	1376^{ab}	1371^{b}	1464^{a}	28.93	0.01
Villus width (µm)	192.5	193.2	192.0	190.7	4.71	0.90
Crypt depth (µm)	154.2	156.5	161.2	167.7	4.10	0.23
Jejunum						
Villus length (µm)	941.7	954.0	940.5	993.0	36.75	0.72
Villus width (µm)	195.5^{a}	182.2^{b}	178.5^{b}	180.0^{b}	3.90	0.04
Crypt depth (µm)	176.5	181.0	177.5	179.4	4.03	0.87
Ileum						
Villus length (µm)	733.7	755.0	769.2	773.7	20.01	0.52
Villus width (µm)	181.0	193.0	175.5	182.5	4.31	0.08
Crypt depth (µm)	165.0	163.0	167.5	164.0	2.52	0.67

[1] Data represent the mean of 4 broiler chickens per treatment.
The means within the same row with at least one common letter, do not have significant difference (P>0.05).
SEM: standard error of the means.

Table 8 Effects of treatments on intestinal morphology indices in broiler chickens at 42 days of age[1]

Parameters	Treatments				SEM	P-value
	Control	Acidifier (AF)	Toxin binder (TB)	AF + TB		
Duodenum						
Villus length (µm)	1822.7	1849.7	1906.5	1935.4	30.02	0.07
Villus width (µm)	196.5	219.0	200.7	201.5	13.03	0.65
Crypt depth (µm)	235.7^{b}	261.2^{a}	269.5^{a}	283.0^{a}	7.61	0.006
Jejunum						
Villus length (µm)	1175.5	1203.3	1211.2	1208.5	26.11	0.73
Villus width (µm)	229.5	243.5	218.7	253.0	15.01	0.42
Crypt depth (µm)	199.2^{b}	221.0^{a}	231.2^{a}	240.1^{a}	8.03	0.02
Ileum						
Villus length (µm)	1058^{c}	1079^{bc}	1130^{b}	1200^{a}	18.32	0.007
Villus width (µm)	191.2	194.7	198.0	194.2	3.80	0.68
Crypt depth (µm)	170.0^{c}	173.0^{bc}	182.1^{ab}	186.0^{a}	3.35	0.01

[1] Data represent the mean of 4 broiler chickens per treatment.
The means within the same row with at least one common letter, do not have significant difference (P>0.05).
SEM: standard error of the means.

These results are in accordance with the findings of Wang *et al.* (2006) who observed decrease of the serum uric acid in broilers fed dietary toxin binder in their diets. On the other hand, there are reports on the incremental effect of mycotoxins on the serum concentration of uric acid in broilers and laying hens (Swamy *et al.* 2004; Smith *et al.* 2005).

Differences in the serum uric acid levels in the different studies may be due to the type of toxin, type of toxin absorbent, and also the species of birds and even the amount of protein intake.

The results of the present experiment are consistent with the findings of (Ghahri *et al.* 2010) who indicated that the levels of uric acid, total protein, cholesterol, and liver en-

zymes in broiler chickens may be affected by the use of mycotoxin binder.

According to these authors, adding the absorbent of fungal toxins to the diet of broiler chicks increased the serum triglycerides and serum cholesterol and reduced the activity of the liver enzymes. Regarding to these results, Azizpour and Moghadam, (2015) reported that reducing serum cholesterol and triglyceride and increasing liver enzymes activity including ALT and AST are one of the most important signs of liver degeneration in broiler chickens that use aflatoxin contaminated diets.

The experimental treatments had no significant effect on the intestinal microbial population of broiler chickens in the recent trial. These results did not support the findings of Agboola *et al.* (2015) who indicated that the presence of toxin binder in the diet of broiler chicks may increase the bird's performance by controlling the microbial flora of the intestinal tract. In regard to the mechanism of action of toxin binder on the intestinal microbiota activity, no study was found and direct comparison was not made. On the other hand and in contrast with the present findings, there are several reports on the beneficial effects of organic acids (in the diet or drinking water) on the alteration of intestinal microbial counts in broiler chickens (Hashemi *et al.* 2012; Eftekhari *et al.* 2015; Eftekhari *et al.* 2017). The use of acidified drinking water has increased the number of *Lactobacillus* colonies and the reduction in the number of *E. coli* colonies in broiler chickens (Eftekhari *et al.* 2015). The antimicrobial mechanisms of organic acids depend on the physiological conditions of the organism and the physicochemical state of the external environment (Ricke, 2003). In this regard, it has been reported that acidifiers induced their mechanism of action through bacterial cell wall depolarization causing a change in the internal acidity of the bacteria, followed by a change in the rate of nutrient absorption by the bacteria and eventually reducing their activity and reducing the number of colonies (Cherrington *et al.* 1990). The relative weight of the spleen and bursa as lymphoid organs and the antibody titer against Newcastle disease virus (NDV) and IBD were not influenced by the experimental treatments. The results of this study are consistent with the findings of Eftekhari *et al.* (2015) who did not observe any significant changes in the immune function and antibody titer against NDV in broiler chicks that received organic acids in their drinking water. However, our results are not supported by the reports of Emami *et al.* (2013) who observed an improvement in immune response of broiler chickens fed dietary organic acids. On the other hand, our findings regarding the effects of toxin binder on the bird's immune system did not match with the results of Pasha *et al.* (2007).

They reported that using a fungal toxin binder may improve the immunity of broiler chickens. Generally, it can be concluded that the presence of fungal toxins in the diet may prevent the protein synthesis and, consequently, decrease the production of IgA and IgG, and subsequently reduce the production of infections in the birds. Therefore, in challenging conditions, it can be expected that the presence of fungal toxin binders may modulate this mechanism.

The health of the gastrointestinal tract is an important factor affecting the growth of the beneficial bacteria in the gut and subsequently the growth performance of broiler chickens. In most studies on the effect of diet on gut health, intestinal morphological traits are used as the most important tools for investigating the status of the gut health. It is well documented that the absorptive surface may increase in the small intestine by increasing the length of the intestinal villus length in the broiler chickens (Eftekhari *et al.* 2015).

In the present experiment, the use of acidifier in combination with toxin binder improved the intestinal morphological traits of broiler chickens. These results were in parallel with the reports of (Cengiz *et al.* 2012) regarding the positive effect of acidifiers on the intestinal morphology of broiler chicks. It is also indicated that the use of organic acids in drinking water may improve the jejunal morphology of broiler chickens (Eftekhari *et al.* 2015). The mechanism of the action of organic acids on the intestinal morphology was demonstrated by García *et al.* (2007) who reported that the acidifiers decline the intestinal microbial load, which in turn reduces the presence of toxins that are associated with alterations in the intestinal morphology of broiler chickens.

On the other hand, in accordance with the findings of the present research, Agboola *et al.* (2015) observed the improvement of intestinal morphology as a result of the use of fungal toxin absorbent in broiler chicks. It has been reported that fungal toxin binders reduce or prevent the absorption of toxic compounds by the mucosal wall of the gastrointestinal tract.

CONCLUSION

In summary, the results of the present experiment indicated that the experimental treatments did not affect the growth performance, carcass traits, microbial population and immunity of broiler chickens. However, some blood metabolites and liver enzyme activity were influenced by the toxin binder alone or in combination with acidifier. Besides, an improvement in the intestinal morphology was observed in broiler chickens that received acidifier alone or in combination with toxin binder.

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