International Journal of Pharmacology

ISSN 1811-7775

science alert

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Asian Network for Scientific Information

OPEN ACCESS International Journal of Pharmacology

ISSN 1811-7775
DOI: 10.3923/ijp.2016.874.883

Research Article

Growth Performance, Intestinal Histomorphology, Blood Hematology and Serum Metabolites of Broilers Chickens Fed Diet Supplemented with Graded Levels of Acetic Acid

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Abstract

Objective: The present study was performed to determine the influence of dietary Acetic Acid (AA) supplementation on growth performance, intestinal histomorphology, blood hematology and serum constituents of broilers. **Methodology:** A total 200 chicks were randomly divided into five experimental groups with four replicates each (10 chicks/replicate) in a complete randomized design experiment. Treatments were as follow: (AA0: control, AA1: basal diet+0.1% acetic acid kg^{-1}, AA2: basal diet+0.2% acetic acid kg^{-1} and AA3: basal diet+0.3% acetic acid kg^{-1}). **Results:** The results showed that AA supplementation improved weight gain ($p<0.001$) and feed conversion ratio ($p<0.001$). Generally, acetic acid supplementation at 0.3% level improved bird's performance during 2-6 weeks of age. Increased intestinal length and higher intestinal weight were recorded in AA treated birds. Significant reduction in pH of proventriculus and ventriculus ($p<0.01$) was observed with in dose related manner. No significant effects were observed on dressing percentage. Histological observations revealed that intestinal morphology professed positive effects under AA treatment. Furthermore, the effect of dietary AA supplementation was significant ($p<0.05$) only on lymphocytes count and heterophil/lymphocyte ratio. It is obvious that blood of birds fed AA3 diet had the highest concentration of calcium, phosphorous, total protein and globulin comparing with the control diet and other levels of AA. The best results of all parameters were observed in AA3. **Conclusion:** Based on the results, it could be concluded that AA supplementations in feed employs positive effect on performance and intestinal histomorphology of broilers.

Key words: Acetic acid, broiler, histomorphology, hematology, serum metabolites

Received: September 09, 2016 Accepted: October 01, 2016 Published: October 15, 2016

Citation: Zaib Ur Rehman, Ahsan Ul Haq, Naasra Akram, Mohamed E. Abd El-Hack, Muhammad Saeed, Shahid Ur Rehman, Chunchun Meng, Mahmoud Alagawany, Maryam Sayab, Kuldeep Dhama and Chan Ding, 2016. Growth performance, intestinal histomorphology, blood hematology and serum metabolites of broilers chickens fed diet supplemented with graded levels of acetic acid. Int. J. Pharmacol., 12: 874-883.

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Competing Interest: The authors have declared that no competing interest exists.

Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

Birds are usually susceptible to considerable sources of stress during their lifetime in the time immediately after hatching is also a stress period. The digestive tract of chicks which newly hatched is sterile and immature. During this time, chicks are exposed to pathogenic microorganisms[1]. So, poultry feed is routinely supplemented with the antibiotics to protect birds from pathogenic microorganisms and also as growth promoters (AGP) to enhance the performance and reduce the population of the potentially pathogenic bacteria[1,2]. Continues use of antibiotics in the poultry feed leads to microbial resistance and these resistance are transmitted to human microbiota[3,4]. Aforementioned bacterial resistance and cross-resistance to antibiotics is progressively considered as a peril for the animal and human health[4]. Thus, the European Union announced plans to ban the use of ABGP in feed[5]. There are a dire need to find alternatives to AGP. A number of feed additives such as probiotics, prebiotics, symbiotic, herbal growth promoters and Organic Acids (OA) are used as alternatives to ABGP having number of favorable effects like control of pathogenic microorganisms and to enhance the growth of beneficial microorganisms. Organic acids are promising alternatives amongst these additives[6]. Supplementation of organic acids in feed improves the growth performance by enhancing the nutrient absorption and decrease the population of pathogenic bacteria in the digestive tract[7,8]. Short chain organic acid like Acetic Acid (AA), propionic acid and butyric acid are well known for their role in the energy metabolism. Particularly, AA which is present in the form of acetyl-coenzyme A in cells and is vital for the ATP production and biosynthesis of long chain fatty acids[9]. Acetic acid supplementation in diet improves the growth performance[10], quicken intestinal epithelial cell division, increases weight of intestinal tissue, resulting in changes in villus width, height and area of the duodenum, jejunum and ileum, which all are the promising factors for the better intestinal health and nutrient digestion and absorption[11]. Most of the studies have compared the efficacy of different organic acid or with antibiotics. But, the studies about the effect of different levels of AA on intestinal health are limited. Moreover, albeit short chain fatty acids like acetic acid and propionic acid have been used successfully as sanitizers for water, there is still little available information about using acetic acid in poultry diets. Therefore, the present study was designed to recommend the optimum level of dietary acetic acid supplementation in broiler feed to obtain the best growth performance, carcass characteristics, intestinal pH, histological changes in the duodenum, jejunum and ileum as well as optimal blood constituents.

MATERIALS AND METHODS

The current study was conducted at Poultry Research Center, University of Agriculture, Faisalabad, Pakistan in collaboration with the PMAS-Arid Agriculture University, Rawalpindi. All procedures of the experiment were carried out according to the Local Experimental Animal Care Committee and approved by the ethics of the institutional committee of University of Agriculture, Faisalabad, Pakistan.

Design, birds, diets and management: One hundred and eighty days old, locally purchased, Hubbard classic broiler chicks were reared until 1 week. Later, 120 middle weight (152 ± 2.6 g) birds were randomly placed in four experimental groups (AA0, AA1, AA2 and AA3) with 3 replicas each in a complete randomized design experiment. Group AA0 served as control i.e., without any supplementation. However, group, AA1, AA2 and AA3 were supplemented with AA at 10, 20 and 30 g kg^{-1} of feed, respectively. Birds in different groups were fed experimental diet from 8-42 days of age, starter diet for 8-28 days and finisher diet for 29-42 days of age. Diets were formulated to be iso-caloric and iso-nitrogenious according to NRC[12] as shown in Table 1. Acetic acid used in current

Table 1: Ingredients and nutrient composition of the basal starter and finisher diets (g kg^{-1})

Items	Starter (0-28 days)	Finisher (29-42 days)
Ingredients (g kg^{-1})		
Maize corn	536.89	563.34
Rice polishing	150.00	150.00
Soybean meal	122.87	100.00
Rape seed meal	40.00	40.00
Sunflower meal	40.00	40.00
Cotton seed meal	40.00	40.00
Fish meal	48.70	41.00
Marble chips	11.50	12.71
Monohydrated di-calcium phosphate	2.62	3.96
Manganese sulfate	0.09	0.08
Zinc sulfate	0.01	0.01
Vitamin premix*	1.00	1.00
Salt	1.01	1.43
Sodium Bi-carbonate	1.00	1.00
Lysine sulfate	2.03	2.39
DL-methionine	1.14	1.70
L-threonine	0.14	0.38
Phytase**	1.00	1.00
Total	1000	1000
Calculated analysis* **		
CP (%)	23.00	21.00
ME (kcal kg^{-1} diet)	2951	3099
Ca (%)	1.00	0.90
P (Available) (%)	0.45	0.40
Lysine (%)	1.20	1.05

*Vitamin premix: Each 2.5 kg consists of: Vit A: 12000, 000 IU, Vit D3: 2000, 000 IU, Vit E: 10 g, Vit k3: 2 g, Vit B1: 1000 mg, Vit B2: 49 g, Vit B6: 105 g, Vit B12: 10 mg, Pantothenic acid: 10 g, Niacin: 20 g, Folic acid: 1000 mg, Biotin: 50 g, Choline chloride: 500 mg, **Phytase and ***Calculated according to NRC[12] experiment was purchased from Ittehad Chemicals Ltd., Pakistan. Different levels of AA were sprayed and mixed in the feed. All replicates had *ad libitum* access to fresh and clean water throughout the trial period. The chicks were raised in a room having 12 pens, having uniform size (3×4), which were white washed and disinfected before the start of experiment. Wood shaving layer of 3 inches was used as litter in each pen. The litter material was stirred regularly during the trial to keep it in a dry condition. Birds in all replicates were reared under the identical management conditions e.g., temperature, relative humidity, light, ventilation and floor space. All birds were vaccinated against Newcastle Disease (ND) at the age of 5 and 18 days and Infectious Bursal Disease (IBD) at the age of 8, 18 and 28 days.

Investigated measurements

Growth performance: Performance data i.e., Feed Consumption (FC), Weight Gain (WG) and Feed Conversion Ratio (FCR) were collected at 28 and 42 days. At the age of 28 days, all the chicks in each replicates were weighed by an electronic weighing balance to measure the average body weight (g). The feed consumed by the chicks in each replicate was measured on 28 and 42 days. Feed conversion ratio [FCR, g g^{-1} (g b.wt., gain g^{-1} of feed consumed)] for each replicate was determined.

Carcass and intestinal histomorphology: At end of trial, three birds from each replicate were randomly selected, individually weighed and slaughtered to investigate the following parameters, dressed weight and pH, weight, length and histomorphology of the different segments of the intestine. A digital pH meter was employed to measure pH of diverse parts of the gastrointestinal tract according to the method described by Al-Natour and Alshawabkeh[13]. Intestinal histomorphological of slaughtered birds were studied according to the method of Adil *et al.*[7].

Blood parameters: A blood sample of 5 mL was collected by wing vein puncture from three birds per replicate by using 24-guage needle in separate screw type tubes for obtaining serum. These blood samples were centrifuged in a centrifuge machine for 5 min at 2000 rpm. Serum samples were taken out of the centrifuge tubes and stored in refrigerator at -4°C until analysis. The serum samples were analyzed for its concentrations of calcium (mg dL^{-1}), phosphorus (mg dL^{-1}), total protein (g dL^{-1}), albumin (g dL^{-1}) globulin (g dL^{-1}) by kit method. Antibody titer for gumboro (IBD) was determined by using technique as described by Akiba *et al.*[14].

Statistical analysis: Collected data were studied by statistical analysis and interpretation was done using ANOVA technique with completely randomized design employing SPSS 16.0. Duncan multiple range test was used to compare means of the treatments[15].

RESULTS AND DISCUSSION

Growth performance: The effects of AA inclusion in broiler feed on performance of birds are shown in Table 2. From 2-4 weeks of age the highest Weight Gain (WG) was recorded in birds fed diet AA3 and AA1 ($p<0.05$) as compared to control group. It is obvious that the highest ($p<0.05$) WG was recorded by AA3 and AA2 from 5-6 week of age; however, lowest WG was observed in the control group (AA0). It is worth noting that increasing AA level was associated a significant ($p<0.05$) gradual increase in WG with all studied periods. Feed consumption was significantly ($p<0.01$) impacted due to dietary AA supplementation only at the first period (2-4 weeks of age). The highest FC ($p<0.01$) was recorded by the control group, while the lowest ($p<0.01$) was noticed in the group fed on diet supplemented with the highest AA level (AA3). A gradual improvement in FCR was observed with increasing levels of AA during starter ($p<0.01$), finisher ($p<0.01$) and overall ($p<0.01$) phases.

Carcass and intestinal histomorphology: The dietary AA supplementation significantly ($p<0.01$) affected the pH of the gizzard and proventriculus. The pH of gizzard and

Table 2: Effect of the acetic acid supplementation on body weight gain, feed consumption and feed conversion ratio of broilers

	2-4 weeks			5-6 weeks			2-6 weeks		
Treatments	WG	FC	FCR	WG	FC	FCR	WG	FC	FCR
AA0	891.80^{c}	1695.0^{a}	1.90^{a}	1068.0^{c}	2381.5	2.23^{a}	1959.8^{c}	4057.3	2.07^{a}
AA1	935.75^{ab}	1689.3^{a}	1.80^{b}	1142.6^{b}	2392.8	2.09^{b}	2078.4^{b}	4082.0	1.96^{b}
AA2	923.98^{b}	1642.4^{b}	1.78^{b}	1194.8^{a}	2403.3	2.01^{bc}	2118.8^{a}	4015.7	1.89^{bc}
AA3	947.25^{a}	1628.8^{b}	1.72^{c}	1200.9^{a}	2363.0	1.96^{c}	2148.1^{a}	3941.8	1.83^{c}
SEM	6.60	9.88	0.020	16.72	7.44722	0.03249	21.99	24.98	0.02873
p-value	0.00	0.006	0.00	0.00	0.278	0.001	0.000	0.214	0.001

^{a,b,c}Column means with different superscripts differ significantly at $p<0.05$

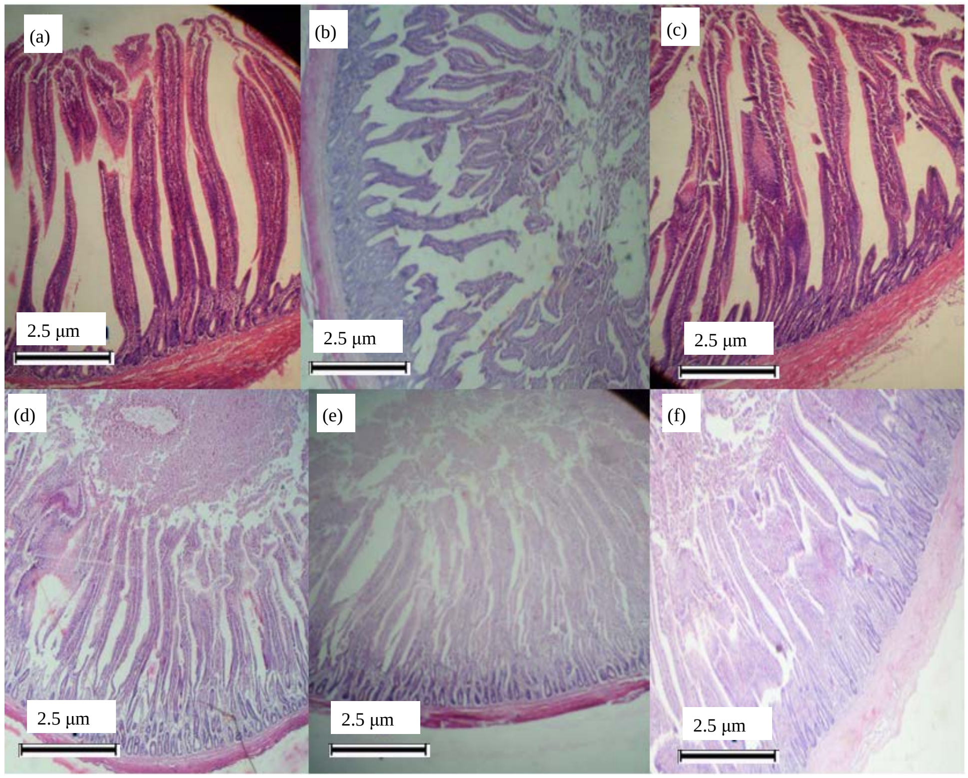

Fig. 1(a-f): Photograph of different parts of small intestine elaborating the dietary supplementation of acetic acid on histomorphology of organ. Control group AA0 is without any supplementation, (a) Duodenum, (b) Jejunum, (c) Ileum and AA1 group is supplemented with 0.1% AA, (d) Duodenum, (e) Jejunum and (f) Ileum, X100 (H and E)

Table 3: Effect of the acetic acid supplementation on the on the pH, length and weight of the intestine and dressing percentage of broilers

	pH of different segments of the intestine							
Treatments	Proventriculus	Gizzard	Duodenum	Jejunum	Ileum	Length of the small intestine (cm)	Weight of the intestine (U)	Dressing (%)
AA0	3.37[a]	2.51[a]	5.36	6.28	6.28	174.50[c]	43.60[c]	68.33
AA1	3.29[ab]	2.36[b]	5.37	6.34	6.31	177.00[bc]	46.07[bc]	68.23
AA2	3.20[bc]	2.36[b]	5.43	6.29	6.28	180.00[b]	49.20[b]	68.67
AA3	3.14[c]	2.22[c]	5.38	6.32	6.29	184.67[a]	53.73[a]	68.67
SEM	0.03	0.04	0.02	0.02	0.02	1.21	1.21	0.24
p-value	0.002	0.01	0.63	0.82	0.97	0.00	0.00	0.92

[a,b,c]Column means with different superscripts differ significantly at p<0.05

proventriculus was maximum in AA0 group and minimum in AA3 (Table 3). The pH of duodenum, jejunum and ileum was not influenced by the dietary treatments. Supplementation of the AA caused a piecemeal increase ($p<0.01$) the length and weight of the intestine (Table 3). There was no any significant ($p>0.05$) effect of treatments on the dressing percentage. Results of the histomorphometric examination and photomicrographs of the intestines of each group are shown in the Table 4 and Fig. 1 and 2 respectively. The obtained results indicated that villus heights of the duodenum, jejunum and ileum were significantly ($p<0.01$) impacted by the supplementation of the AA (Table 4). Villus height was recorded as maximum in the group fed with the diet containing 30 g AA kg^{-1} diet. Crypt depth of the duodenum and ileum increased in treatments AA2 and AA3 compared with others (Fig. 1). However, the different AA levels did not affect the crypt-depth of the jejunum (Table 4, Fig. 1). Villus surface area of duodenum and jejunum was also increased ($p<0.01$) by the AA supplementation.

Blood hematology: Table 5 showed that the effect of dietary AA supplementation was significant ($p<0.05$) only on lymphocytes count and heterophil/lymphocyte ratio (H/L ratio). The highest lymphocyte percentage values were

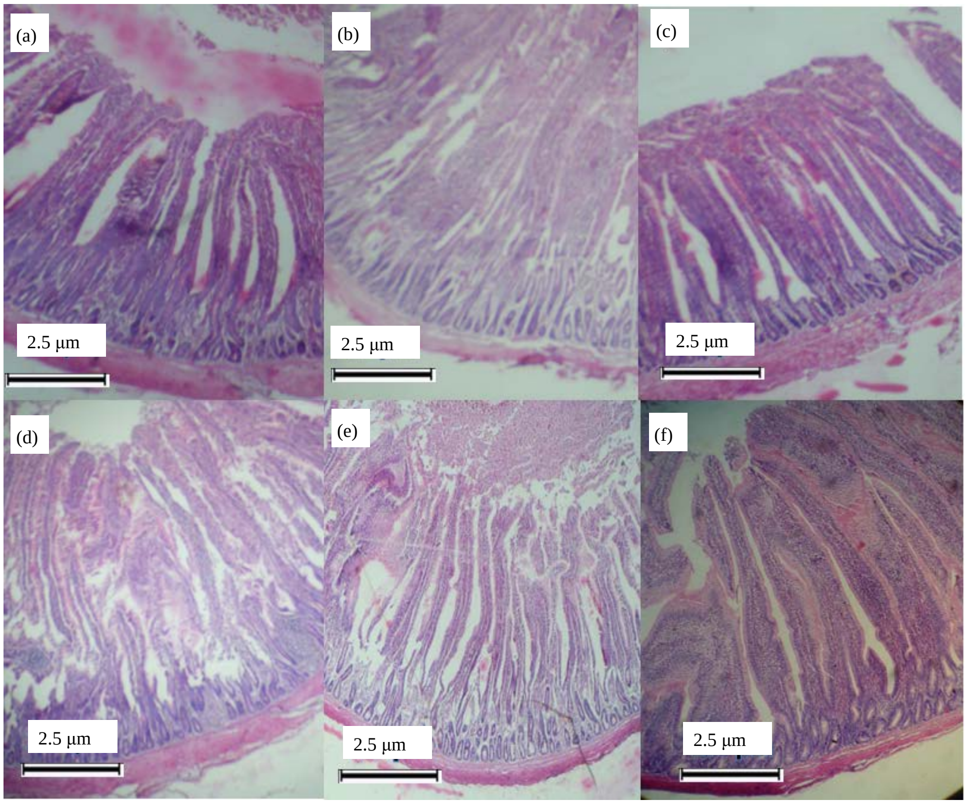

Fig. 2(a-f): Photograph of different parts of small intestine elaborating the dietary supplementation of acetic acid on histomorphology of organ. Group AA2 = 0.2% AA supplementation, (a) Duodenum, (b) Jejunum, (c) Ileum and AA3 group = Supplemented with 0.3% AA (d) Duodenum, (e) Jejunum and (f) Ileum, X100 (H and E)

Table 4: Effect of acetic acid supplementation on histomorphology of small intestines of broilers

	Duodenum			Jejunum			Ileum		
Treatments	Villus height (µm)	Crypt depth (µm)	Villus surface area (mm^2)	Villus height (µm)	Crypt depth (µm)	Villus surface area (mm^2)	Villus height (µm)	Crypt depth (µm)	Villus surface area (mm^2)
AA0	1309.00^b	207.67^b	0.2400^b	121.80^c	189.00	0.2000^b	733.00^c	144.33^c	0.1200
AA1	1332.00^b	236.67^a	0.2767^{ab}	124.97^c	212.00	0.2367^{ab}	779.67^b	146.00^c	0.1267
AA2	1368.70^a	230.00^a	0.2900^a	135.17^b	230.00	0.2500^a	807.00^{ab}	170.00^b	0.1233
AA3	1379.70^a	229.33^a	0.3167^a	147.43^a	218.00	0.2767^a	828.00^a	194.33^a	0.1367
SEM	9.29	429.49	0.01	32.59	6.26	0.01	11.78	6.60	0.00
p-value	0.001	0.058	0.023	0.001	0.106	0.023	0.002	0.001	0.427

[a,b,c]Column means with different superscripts differ significantly at $p<0.05$

Table 5: Effect of acetic acid supplementation on the blood hematology of the broilers

Treatments	RBCs (Million mm^{-3})	WBCs (Thousand mm^{-3})	Heterophill (%)	Lymphocyte (%)	H/L ratio	PCV (%)	Hb (mg/100 mL)	MCV	MCH	MCHC
AA0	2.34	25.70	25.28	66.05^b	0.38^a	35.87	17.00	153.72	72.81	47.40
AA1	2.35	25.37	25.35	68.43^{ab}	0.37^{ab}	35.80	16.00	152.62	68.22	44.71
AA2	2.38	25.80	25.26	70.67^a	0.36^b	36.27	16.73	152.64	70.35	46.09
AA3	2.33	25.63	25.27	70.80^a	0.35^b	35.57	16.57	152.50	70.98	46.60
SEM	0.02	0.23	0.04	0.68	0.00	0.25	0.25	1.56	0.97	0.62
p-value	0.88	0.94	0.86	0.01	0.01	0.84	0.62	0.99	0.46	0.54

[a,b,c]Column means with different superscripts differ significantly at $p<0.05$

found in treatment AA3 followed by AA2, AA1 and AA0, respectively. Lymphocyte percentage was significantly increased from 66.05-70.80% by the addition of acetic acid. Acetic acid supplementation showed decreasing trend of

Table 6: Effect of the acetic acids on the serum constituents and antibody titer of the broilers

Treatments	Calcium (mg dL^{-1})	Phosphorus (mg dL^{-1})	Total protein (g dL^{-1})	Albumin (g dL^{-1})	Globulin (g dL^{-1})	A/G ratio (g dL^{-1})	IBD antibody titer
AA0	8.10^{b}	6.78^{c}	3.43^{b}	1.82	1.70^{d}	1.07^{a}	3196.33^{c}
AA1	8.33^{b}	7.17^{b}	4.66^{a}	1.83	1.87^{c}	0.98^{a}	3824.67^{b}
AA2	9.40^{a}	7.30^{ab}	4.65^{a}	1.80	2.20^{b}	0.82^{b}	4480.67^{a}
AA3	9.60^{a}	7.40^{a}	4.85^{a}	1.81	2.38^{a}	0.76^{b}	4746.00^{a}
SEM	0.21	0.07	0.17	0.02	0.08	0.04	193.39
p-value	0.00	0.00	0.00	0.91	0.00	0.00	0.00

^{a,b,c}Column means with different superscripts differ significantly at $p<0.05$

H/L ratio. Percentage of H/L ratio decreased from 0.38-0.35% by the addition of acetic acid.

Blood metabolites and immunity: Results in Table 6 showed statistical ($p<0.01$) impacts on all blood parameters studied except albumin percentage due to dietary AA supplementation. It is noticeable that blood of birds fed AA3 diet had the highest content of Ca, P, total protein and globulin comparing with the control diet and other levels of AA. While, the lowest values of the aforementioned metabolites were found in serum of birds fed the control diet. In a converse trend, the highest A/G ratio observed in AA0 group and the lowest was found in the group of AA3. For IBD antibody titer, the group of AA2 excelled ($p<0.01$) the other experimental groups and recorded the highest value of IBD antibody titer being 4480.67 vs. the lowest value (3196.33) which recorded by the control (AA0).

Growth performance: Supplementing broiler feed with various organic acids has positive effect on growth performance and feed efficiency[16]. Hudha *et al.*[10] revealed that the use of acetic acid as dietary supplementation in broiler diets improved growth performance traits. Mustafa *et al.*[17] found that adding AA (1 and 2%) in the drinking water of broiler chicken enhanced the values of live body weight at 42 days of age. Consisting with results reported by Afsharmanesh and Pourreza[18]. These acidifiers possibly improve the growth by refining digestion through numerous mechanisms. They are supposed to increase gut health by encouraging the growth of beneficial bacteria and suppressing the pathogenic microbes by reducing pH and buffering capacity of feed[19]. A better buffering ability of feed is anticipated to back off the multiplication and/or colonization of undesirable microbes in the GIT[20]. Natural acids can likewise empower pancreatic discharges[21], which improves the absorbability, assimilation and retention of amino acids and minerals[22,23]. Proper digestibility and availability of nutrients to broiler birds may leads to more WG and better FCR. Seifi *et al.*[24] postulated that the addition of 0.5% acetic acid in broilers diet improved FCR; which is consisted with those reported by Chowdhury *et al.*[25].

Results of the present study are in line with some previous studies showing that organic acid supplementation in broiler diet increases WG[16,26]. Proposed mechanism of improving weight gain may either be due to the fact that organic acids reduce the growth of many feed and intestinal microbes, consequently, reduce intestinal colonization and infectious inflammatory processes at mucosa[27], which decrease the thickness of mucosa layer, enhance height and secretion of villus that leads to improvement in weight gain of broilers. Contradictory results were found in different study that supplementing organic acid to broilers diet did not affect the weight gain[28]. Variations in results may either be due to variation in the level of acids used, variation in specific acid and variation in feed ingredients or environmental conditions. In the contrary, Pinchasov and Elmalich[29] and Islam *et al.*[30] found depressed body weight gain in birds fed diets supplemented with acetic acids. Seifi *et al.*[24] found no significant effects of dietary acetic acid on body weight of broiler chickens.

Our results revealed insignificant effect of AA supplementation on dressing percentage of broilers. Similar to our results, Islam *et al.*[30] confirmed no significant effect of acetic acid on dressing percentage of broiler chickens. Consistent with Hamano and Kurimoto[9] who supplemented acetylated wood powder in broiler diet and reported insignificant impact on dressing percentage. Contrary to our results, Hudha *et al.*[10] reported an improvement in dressing percentage of broilers when they were offered acetic acid in drinking water. In this study, acetic acid supplementation significantly ($p\leq 0.05$) decreased pH of the proventriculus and gizzard and had no significant effect on pH of the duodenum, jejunum and ileum. Butyric acid supplementation at 0.2, 0.4 and 0.6% dose level significantly ($p\leq 0.05$) depressed the pH of crop, proventriculus and gizzard as compared to control[31], which is similar to this current study.

Carcass and intestinal histomorphology: The results of intestinal histomorphology are in harmony with Jongbloed *et al.*[23] findings which reported more ileum villus height in chickens fed on diets enriched with organic acid salts than in those fed with diets depleted in mannose oligosaccharide. Similar findings are found by Paul *et al.*[32] who used organic acid salts at 3 mg kg^{-1} feed. Results of the latter authors showed significant ($p\leq 0.05$) improvement in the intestinal villus height. In this context, Garcia *et al.*[33] claimed that broiler chickens fed formic acid had the greater villus height, width and crypt depth compared to control group. Results of the present study revealed an increase in length and weight of small intestine. Similarly, Denli *et al.*[34] concluded that the supplementation of the diet with the antibiotic, probiotic and organic acid increased intestinal weight and length at day 42 of age. Mustafa *et al.*[17] found significant improvements ($p<0.05$) in villi height of jejunum, ileum and duodenum at 42 days of age when drinking water enriched with acetic acid (1 and 2%) comparing with the control. Authors found also a significant increase ($p<0.05$) in crypt depth of ileum and jejunum in the treatment group drunk water with 2% acetic acid as compared with control group. Results of Celik *et al.*[35] are also in agreement with present study results demonstrating that supplementation of the probiotic and OA to feed increased the weight of the small intestine. Kum *et al.*[11] theorized that organic acid supplementation significantly ($p\leq 0.01$) increases the villus width, height and area of the duodenum, jejunum and ileum of male broilers. Leeson *et al.*[26] compared the butyrate with the bacitracin; results assured an increase in crypt depth in duodenum of broiler chicks fed 0.2% butyric acid. This increase in villus height, area and crypt depth of the intestine may be due to the antimicrobial properties of the organic acid which lowered the number of pathogenic bacteria in the intestine. These bacteria alter the permeability of this natural fence[27] aiding the invasion of the pathogens and hurtful substances leading to inflammatory processes at the intestinal mucosa[36]. Xia *et al.*[37] stated that most of organic acidifiers added to diet induced a significant increase of the intestinal villus height in chickens. Feed acidifiers have direct stimulatory impact on the gastro intestinal cell proliferation linked with short chain fatty acids. It is believed that short chain unsaturated fats build plasma glucagon-like peptide 2 and ileal pro-glucagon mRNA, glucose trans expression and protein appearance, which are all signs which can possibly intercede gut epithelial cell multiplication[38].

Blood hematology: Results of this study indicated that acetic acid only significantly affect the lymphocyte and H/L ratio. There was non-significant effect on the red blood cells, white blood cells, heterophil, packed cell volume, hemoglobin, mean corpuscular volume, mean corpuscular hemoglobin and mean corpuscular hemoglobin concentration. Results regarding increase in lymphocytes are in line with Wang *et al.*[39]. The aforementioned authors used phenyllactic acid in the broiler diet and found an increase in white blood cells which is contrary to the present study. Authors also reported an increase in lymphocyte which is in agreement to our findings. Wang *et al.*[40] studied the effect of different levels of phenyllactic acid on red blood cells, white blood cells, lymphocytes in layer diet and results indicated an increase in lymphocyte. Citric acid supplementation enhanced the density of the lymphocytes in the lymphoid organs so, enhanced the non-specific immunity[41]. Birds having the greater density of lymphocytes have stronger immune status to combat antigens[42]. Khosravi *et al.*[43] mentioned that organic acids supplementation do not have any significant effect on the erythrocyte, leukocyte, eosinophil, heterophil and lymphocyte of broilers which is in line to the current finding.

Blood metabolites and immunity: Dietary supplementations with different levels of acetic acid significantly ($p\leq 0.01$) increased the serum total protein and globulin and significantly decreased the A/G ratio. These results are supported by those reported by Abdel-Fattah *et al.*[44] who used the acetic and lactic acid and observed a significant ($p\leq 0.05$) improvement in serum globulin and decrease in A/G ratio. Globulin is a source of antibody production, so its level in the serum is a good indicator of immune responses and consequently better disease resistance[45]. Dietary acidification increases the serum globulin in broilers, which also had better immune responses[46]. Organic acid supplementation increased the serum globulin so, enhanced specific immunity (antibody production). Acetic acid supplementation significantly increased serum calcium and phosphorus. These results are in agreement with Brenes *et al.*[47]. Many studies showed that organic acids increased phytate P utilization by poultry[48,49]. Adil *et al.*[50] used butyric acid, fumaric acid and lactic acid in the broiler diet and the results indicated a significant increase in serum concentration of calcium and phosphorus. This increase in the serum calcium and phosphorus may be due to the Ca-complexing property of the organic acid. Anionic parts of the organic acids form a complex with the cations so, enhances the bioavailability and ultimately leads to high serum values of the calcium and phosphorus.

Results of the present study showed an increase in antibody titer against. These results are confirmed by those mentioned by Das *et al.*[51] who used organic acid and reported increased antibody titer against Newcastle disease vaccine. Exact mechanism by which the organic acid affects the antibody titer is not clear but it is proposed that organic acid supplementation affect the microorganisms and increase the immunocompetent cells[52,25] that are responsible for the development of the gut immune system. Organic acids also enhance the bioavailability of different minerals like Zn[47], Fe, Ca and P, which are important to immunity[53]. Saleem *et al.*[54] proved that supplementing broiler diet with 0.1% acetic acid improved the immune response and growth performance traits.

CONCLUSION

From the aforementioned the dietary supplementation of AA can improve WG, FCR, length of small intestine, weight of small intestine, villus height, crypt depth and villus surface area of duodenum, jejunum and ileum. Consequently it could be suggested that acetic acid supplementation in broiler feed can be used to improve performance and boost immunity. This approach is destined to make poultry industry resistant against intestinal ailments and food pathogens.

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