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Original Article

Effects of the Inclusion of Dietary Organic Acid Supplementation with Anti-Coccidium Vaccine on Growth Performance, Digestibility, Fecal Microbial, and Chicken Fecal Noxious Gas Emissions

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ABSTRACT

A total of 792 conventional healthy 1-day-old Ross 308 broilers chicks (mixed gender) with average body weight of 42.30±1.14 g (mean ± SD) were used in the experiment, which lasted for 35 days. Chicks were randomly allotted into one of four treatment diets, each one having 11 replicate cages with 18 birds each, being fed corn-soybean meal (SBM) based diets. Dietary treatments were CON (basal diet with unvaccinated birds); OA = CON + 0.1% organic acids; ACB = CON + anti-coccidium vaccine; OAACV = CON + 0.1% organic acid + anti-coccidium vaccine. Significant results were observed, with improved body weight (p=0.059; 0.064; 0.034) during days 1-7, 8-21, and overall, respectively. Significant effects were also observed on the feed conversion ratio (p=0.037) through the overall experiment, with no effects on feed intake on OAACV as compared to other treatment groups. Total track digestibility of dry matter (p=0.049) improved significantly in the OAACV treatment group. Additionally, beneficial effects were observed in the OAACV treatment group, with improvements in fecal microbial population (increased *lactobacillus*) and reduced NH_3 gas emissions. Broilers fed the OAACV treatment tended to display reduced drip loss in the meat samples analyzed on days 5 and 7 (p=0.067, 0.072). In summary, our findings revealed that dietary inclusion of organic acid supplementation with anti-coccidium had a beneficial effect on broilers affected by coccidian infection, also improving growth performance, digestibility, fecal *Lactobacillus* counts, and reducing NH_3.

INTRODUCTION

Coccidiosis is one of the familiar protozoan gastrointestinal diseases that have negative effects on poultry production. Moreover, it affects domestic animals with mono-celled parasites of the *Eimeria* genus, commonly referred to as coccidia (Peek & Landman, 2011). This parasite can spread from one animal to another via infected feces in the oocyst stage or through the ingestion of sporulated oocysts in the environment (Upadhaya *et al.*, 2019). After ingestion, broilers' digestive tracts stimulate trophozoite of the oocyst in the liver and trigger the release of sporozoites, which assail and ruin colon mucosa cells. As a result, animals may exhibit decreased feed intake, bloody diarrhea, and reduced body weight (Dalloul & Lillehoj, 2005; Gilbert *et al.* 2011). Besides, these parasites can affect broilers' meat quality and growth performance, also raising susceptibility to ancillary infections (Nagi & Mathey 1972). In addition to these symptoms, *Eimeria* infections have been shown to reduce bone mineralization (Sakkas *et al.*, 2017). Various antiseptic medicines have been used against coccidian oocysts, such as methyl bromide, carbon disulfide, and ammonia (Williams, 1997). However, these antiseptics' residues in poultry products cause harmful disease in consumers. Thus, the global poultry industry has been urged to prevent the emergence of drug resistance and pursue alternative, more effective methods to control broilers' coccidiosis (Dalloul & Lillehoj, 2005; Williams, 2005; Abbas *et al.*, 2011). Furthermore, resistance against the anti-parasite drug used for oocyst infections has become a huge issue in commercial poultry industry, increasing its production cost. Anti-coccidial vaccination has thus become the best alternative and has been widely used in the prevention of coccidiosis infection in broilers (Chapman, 2000; Williams, 2002). *Eimeria* species, such as *E. tenella, E. acervulina*, and *E. maxima*, are commercially used as cocci-vaccines containing viable oocysts (Dalloul & Lillehoj, 2005). However, Chapman *et al.* (2002) reported that live vaccination causes growth reduction and leads to secondary enteritis. Moreover, Levine (1939) reported that sulfonamide has been used to cure coccidiosis in chickens.

Many researchers have recently focused on naturally developed alternatives to maintain the efficiency of broiler production (Abbas *et al.*, 2011; Hayajneh, 2019), and the present study supports this research direction. Dibner & Buttin (2002) emphasized that organic acids are potential antibiotic alternatives, as they have the ability to improve chickens' performance by changing the pH of the intestinal tract. Moreover, organic acids facilitate the protection of broilers from pH-sensitive pathogens and enrich their immune system physiology. Zhao *et al.* (2013) found that the dietary addition of organic acids and prebiotics at different levels had a positive effect on broilers' growth performances. However, limited investigation was performed on the influence of organic acid supplementation with anti-coccidian vaccination in poultry. Thus, the aim of this research was to evaluate the efficacy of organic acid supplement with anti-coccidium vaccine on growth performance, nutrient digestibility, fecal microbial, fecal noxious gas emission, meat quality, and intestinal villi on chickens.

MATERIAL AND METHODS

The experimental protocol was reviewed and approved by the Animal Care and Use Committee of Dankook University (Animal Ethics Approval Number: DK-1-1943). Organic acids were provided by Morningbio Co., Ltd., Cheonan, Republic of Korea.

Trial design and diets

A total of 792 conventional healthy 1-day old Ross 308 broiler chicks (mixed gender) with average BW of 42.30±1.14 (mean ± SD) were used in the experiment, which lasted for 35 days. Chicks were randomly allotted into one of four treatment diets, each one having 11 replicate cages with 18 birds each, being fed corn-soybean meal (SBM) based diets. The dietary treatments were: CON (basal diet with unvaccinated birds); OA = CON + 0.1% organic acid; ACV = CON + anti-coccidium vaccine; OAACV = CON + 0.1% organic acid + anti-coccidium vaccine. Chickens were grown in a room at 33±1 ºC for the first 3 days and the temperature was reduced gradually until 24 ºC, maintaining humidity around 60% for the rest of the experiment. Stainless steel cages (1.75 × 1.55 m) of identical size were used for chickens' housing, with free access to feed and water. The basal diet was formulated as per NRC's (2012) guideline requirements and provided to broilers during the starter (days 0-7), grower (8-21), and finisher (d22-35) phases (Table 1). Anti-coccidium vaccine was administered at the end of the 1st week and vaccines were prepared using BIOPHARM's LIVACOX®T product, with 1 ml of vaccine diluted in 100 ml, diluted orally and administered at 4 ml per dose.

Table 1 – Broilers' feed composition (as fed-basis).

Item	Starter	Grower	Finisher
Ingredients (%)			
Corn	43.63	47.45	53.78
Soybean meal	35.08	31.28	28.18
Corn gluten meal	13.00	13.00	10.00
Wheat bran	3.00	3.00	3.00
Soy oil	1.76	1.74	1.51
TCP	1.81	1.81	1.81
Limestone	0.94	0.94	0.94
Salt	0.36	0.36	0.36
Methionine (99%)	0.19	0.19	0.19
Lysine	0.03	0.03	0.03
Mineral mix[1]	0.10	0.10	0.10
Vitamin mix[2]	0.10	0.10	0.10
Total	100.00	100.00	100.00
Analyzed value			
Crude protein, %	23.00	21.50	20.00
Ca, %	1.10	1.08	1.07
P, %	0.83	0.82	0.79
Available P, %	0.54	0.53	0.52
Lys, %	1.26	1.15	1.06
Met, %	0.54	0.52	0.50
ME, kcal/kg	3200	3200	3200
FAT,%	4.45	4.51	4.32
Fiber,%	3.55	3.48	3.30
Ash,%	6.76	6.57	6.30

[1] Provided per kg of complete diet: 37.5 mg Zn (as $ZnSO_4$); 37.5 mg Mn (as MnO_2); 37.5 mg Fe (as $FeSO_4 \cdot 7H_2O$); 3.75 mg Cu (as $CuSO_4 \cdot 5H_2O$); 0.83 mg I (as KI); and 0.23 mg Se (as $Na_2SeO_3 \cdot 5H_2O$).

[2] Provided per kg of complete diet: 15,000 IU of vitamin A, 3,750 IU of vitamin D_3, 37.5 IU of vitamin E, 2.55 mg of vitamin K_3, 3 mg of Thiamin, 7.5 mg of Rivoflavin, 4.5 mg of vitamin B_6, 24 ug of vitamin B_{12}, 51 mg of Niacin, 1.5 mg of Folic acid, 0.2 mg of Biotin and 13.5 mg of Ca-Pantothenate.

Chickens' body weight (BW) was measured on days 0, 7, 21 and 35. Feed intake (FI) and feed conversation ratio (FCR) were determined by recording the residual feed on pen basis throughout the trail. Simultaneously, mortality was also recorded for each treatment. 0.20% chromium oxide (indigestible marker) was mixed in broilers' diets for 7 days prior to fecal collection (35th day), to analyze the total track nutrient digestibility of dry matter (DM), nitrogen (N), and energy (E). Excreta samples were randomly collected from at least 4 chickens of each treatment. Samples were then thoroughly mixed and stored in a freezer at -20°C until analysis. All feed and fecal samples were dried and finely grinded to pass through a 1 mm screen sieve. DM and N digestibility are determined using methods established by the Association of Official Analytical Chemists (AOAC, 2000). UV absorption spectrophotometry (UV-1201, Shimadzu, Kyoto, Japan) was used to determine chromium levels in the diets and feces and a Parr 6100 oxygen bomb calorimeter (Parr Instrument Co., Moline, IL, USA) was used to identify energy by measuring combustion heat in the samples. To calculate the apparent total tract digestibility, the following formula was used: digestibility (%) = $\{1- [(Nf \times Cd)/ (Nd \times Cf)]\} \times 100$, where Nf = nutrient concentration in feces (% DM), Nd = nutrient concentration in diet (% DM), Cd = chromium concentration in diet (% DM), and Cf = chromium concentration in feces (% DM).

On day 35, 22 birds per treatment were randomly selected, separately weighed, and slaughtered by cervical dislocation. Breast meat, liver, spleen, gizzard, bursa of Fabricius, and gut fat were then removed by trained personnel. All organs were weighed to express the percentage of body weight. Only breast meats were stored at -20 ºC for further analysis. Longissimus muscle meat color - lightness (L*), redness (a*), and yellowness (b*) - was assessed using a Minolta CR410 (Minolta Co, Japan) chromameter. Simultaneously, a digital pH meter was used to measure pH suspension (Testo205, Testo, Germany). The pH meter probe was measured using two buffers (pH 4.0 and 7.0) and each measurement was repeated three times. Two (2) g of breast muscle samples was used to calculate drip loss value by the Honikel method. The water-holding capacity (WHC) was calculated using the procedure described by Kauffman *et al.* (1986). For this, a 0.3-g sample was taken, placed on a piece of filter paper (125-mm-diameter), and then pressed with 3,000 g of weight for 3 min. The pressed sample's areas and the expressed moisture were measured using a digitizing area-line sensor (MT-10S; M.T. Precision Co. Ltd., Tokyo, Japan). Meat samples were kept in a water bath and cooked at 80°C for 30 min to calculate the cooking loss (Albercht *et al.* 2019).

On the 35th day, 22 broilers per treatment were randomly selected and their fresh excreta samples were collected (2 birds/cage) in micro-tubes, placed in sterile plastic bags, kept in an icebox, and immediately transported to the laboratory for microbial analysis. On the same day, we collect fresh excreta samples from the same birds for gas emission measurements. One gram of excreta sample was diluted in 9 mL of 1 % peptone broth (Becton, Dickinson and Co., Franklin Lakes, NJ) and homogenized. Samples were then serially diluted (10-fold in 1 % peptone solution) and plated onto MacConkey agar plates (Difco Laboratories, Detroit, MI) and *Lactobacilli* medium III agar plates (Medium 638, DSMZ, Braunschweig, Germany) to isolate *E. coli*, *Salmonella* and *Lactobacillus*, respectively. *Lactobacilli* medium III agar plates were incubated in anaerobic conditions at 39 °C for 48 h, and both MacConkey agar plates and *Salmonella Shigella* (SS) agar plates were incubated at 37°C for 24 h. After removing the agar plates from the incubator, *E. coli*, *Salmonella*, and *Lactobacillus* colonies were counted and recorded for statistical analysis.

To measure gas emissions, 300g of fresh excreta samples were placed in 2.6-L plastic boxes with a small hole in the middle of one of its sides and sealed with adhesive plaster. Samples fermented for 24 hours at 25 ºC, with 100 ml of headspace air (approximately 2.0 cm) above the sample, manually shaken for 30 seconds to measure crust formation on the surface, and homogenized. The gas emission concentration was estimated from 5.0 to 100.0 ppm (No. 3La, detector tube; Gastec Corp. Kanagawa, Japan) and from 2.0 to 20.0 ppm (4LK, detector tube; Gastec Corp).

Twenty-two birds from each treatment were randomly picked at the end of the experimental trial and killed by cervical dislocation. Intestinal tract tissue samples were collected from the ileocolic junction (ileum), mid-gut (jejunum), and duodenum regions and placed into neutral buffered formalin for fixation, and intestinal segments were fixed for morphometric analysis and histochemical staining in 10% buffered formalin solutions. Histological experiment samples were performed on 5 µm sections, stained by haematoxylin and eosin, and examined by an Olympus AX70 microscope (Olympus Cooperation, Tokyo, Japan). For each sample, the number of well-oriented intact crypt-villus units was chosen in triplicate for each intestinal cross-section. The classification criteria for villus were based on the intact appearance of lamina propria. Villus length was determined from the tip of the villus to the villus-crypt junction, according to Wilson *et al.* (2018).

Statistical analysis

All data were analyzed as a 2×2 factorial using SAS software's (2000) GLM procedure, and pen was used as the experimental unit. Supplementation of organic acid without vaccine or with vaccine were considered in the model. Results were considered significant at $p<0.05$, and $p<0.10$ was considered a trend.

RESULTS AND DISCUSSION

Coccidiosis has become the most prevalent disease in the poultry industry, resulting in severe economic losses by affecting the developmental output and causing high mortality among young chickens. Several researchers have focused on alternative methods to manage avian coccidiosis, such as adding organic acids to diets (Runho *et al.*, 1997; Thompson & Hinton 1997; Vale *et al.* 2004). Later studies by Upadhaya *et al.* (2019) and Oviedo-Rondon *et al.* (2005) demonstrated that anti-coccidium vaccinated birds fed with feed additive supplementation had improved BWG as compared to birds fed additive supplementation without vaccination. Similarly, in the current study, the group with dietary inclusion of organic acid supplementation and anti coccidium vaccination showed significant improvements in BWG at days 0-7, 8-21, and through the overall experiment (p=0.059, 0.064, and 0034), as compared to other treatment groups. Additionally, FCR was significantly increased during the overall experiment in broilers fed the OAACV treatment group, without effects on feed intake as compared to the CON, OA, and ACV dietary treatments (Table 2). Lee *et al.* (2015) stated that organic acid and medium-chain fatty acids supplementation had improved growth performance in laying hens. Similarly, Li *et al.* (2008) reported that inclusion of 0.5% organic acids blends such as butanoic fumaric and benzoic acid improved growth performance in weanling piglets. To our knowledge, there was limited information available on the combined use of organic acid supplementation and anti-coccidium vaccination in broiler chickens. However, in this study, we observed a significant effect only on BWG, whereas our findings failed to show significant difference on ADFI through the overall experiment. Thus, further research is needed to ascertain the exact reason for the lack of results. Previously, Upadhaya *et al.* (2019) stated that birds vaccinated with anti-coccidium and fed with essential oil blends and vitamin D supplementation showed improved total DM track digestibility as compared to those without feed additive supplementation. In accordance with our study, DM nutrient digestibility was significantly increased (p=0.049) in the OAACV

Table 2 – Effects of organic acid supplementation with anti-coccidium on broilers' growth performance1.

Items	CON	OA	ACV	OAACV	SEM[2]	*p*-value[3]		
						Anti-coccidium	Organic acid	Interaction
Day 1-7								
BWG, g	118	122	117	123	3	0.886	0.643	0.059
FI, g	138	138	138	139	3	0.959	0.938	0.979
FCR	1.182	1.137	1.187	1.130	0.040	0.995	0.235	0.892
Day 8 to 21								
BWG, g	694^{ab}	698^{ab}	685^{b}	723^{a}	11	0.140	0.490	0.064
FI, g	946	958	940	949	17	0.645	0.513	0.938
FCR	1.369	1.326	1.374	1.360	0.028	0.441	0.270	0.583
Day 22 to 35								
BWG, g	882	898	873	890	14	0.543	0.232	0.962
FI, g	1596	1599	1596	1598	23	0.971	0.901	0.983
FCR	1.809	1.781	1.830	1.798	0.022	0.377	0.166	0.932
Overall								
BWG, g	1694^{ab}	1712^{ab}	1676^{b}	1742a	18	0.201	0.752	0.034
FI, g	2680	2696	2673	2686	34	0.813	0.687	0.964
FCR	1.582^{ab}	1.547^{b}	1.570^{ab}	1.597a	0.015	0.203	0.775	0.037
Mortality	5.05	3.54	4.04	5.05	-	-	-	-

[1] Abbreviation: CON, (Basal diet with unvaccinated birds); OA, CON + 0.1% organic acid; ACV, CON + anti-coccidium vaccine; OAACV, CON + 0.1% organic acid + anti-coccidium vaccine.

BWG- Body weight gain; FI- Feed Intake; FCR- Feed conversion ratio

[2,3] Means in the same row with different superscripts differ (p< 0.05). Values represent 11 replicates/treatment, 18 chickens/cage in each of the four treatment groups.

treatment group as compared to other treatment groups (Table 3). Similarly, Stefanello *et al.* (2020) stated that the dietary inclusion of protected organic acids and essential oils significantly increased DM digestibility as compared to challenged control birds. Moreover, Paraskeuas *et al.* (2017) reported that increasing the level of essential amino acids in broiler diets significantly improved DM nutrient digestibility. The growth performance may be mirroring a beneficial effect on nutrient digestibility. However, the improved BWG and FCR of the broiler chickens in the group with dietary inclusion of organic acid supplementation and anti-coccidium vaccination in the present study might be due to the increase in nutrients' digestibility.

Table 3 – Effects of organic acid supplementation with anti coccidium on broilers' nutrient digestibility[1].

Items, %	CON	OA	ACV	OAACV	SEM[2]	*p*-value[3]		
						Anti-coccidium	Organic acid	Interaction
Dry matter	74.80 ^{ab}	76.87 ^{b}	74.87 ^{ab}	77.31 ^{a}	1.42	0.579	0.354	0.049
Nitrogen	73.23	74.56	73.06	74.03	1.18	0.751	0.298	0.868
Energy	75.30	76.33	75.65	75.92	0.75	0.968	0.407	0.624

[1] Abbreviation: CON, (Basal diet with unvaccinated birds); OA, CON + 0.1% organic acid; ACV, CON + anti-coccidium vaccine; OAACV, CON + 0.1% organic acid + anti-coccidium vaccine.

[2,3] Means in the same row with different superscripts differ ($p<0.05$). Values represent 11 replicates/treatment, 18 chickens/cage in each of the four treatment groups.

Intestinal health is one of the key factors determining bird efficiency, and hence the economics of poultry production, and intestinal microflora plays an important role in intestinal health (Paul *et al.*, 2007). Fecal microbes play an important role in preventing the colonization by pathogens, detoxifying harmful substances, recycling nitrogen, contributing with microorganism vitamins' synthesis, degrading some carbohydrates, and aiding the absorption of additional nutrients (Clench & Mathias 1995). An excess of coliform bacteria in the gastrointestinal system causes diarrhea, leading to a decline in the growth performance of domesticated animals. Previously, Yang *et al.* (2019) demonstrated that organic acid supplementation had increased *Lactobacillus* population and decreased *E. coli* population in weanling pigs. In this study, the OAACV treatment group had significantly improved *Lactobacillus* counts as compared to the CON, OA, and ACV treatment groups. However, the *E coli* count was not affected ($p>0.05$) in any of the treatments (Table 5). Likewise, Nguyen & Kim (2020) reported that birds fed an organic acid supplementation showed an increased *Lactobacillus* count. Kaper *et al.* (2004) stated that the increase in *Lactobacillus* counts in the intestinal tract had a positive effect on gut health. Therefore, we assumed that the OAACV treatment had a beneficial effect on poultry gut microflora. According to Okali *et al.* (2007), NH_3 and H_2S are the main elements of pig manure contributing to air pollution. Yan *et al.* (2011) suggested that fecal gas emissions are related to nutrient digestibility, since higher digestibility levels produce less substrate for microbial fermentation in the large intestine, reducing fecal noxious gas emissions. Accordingly, NH_3 gas emissions were significantly reduced in broiler chickens fed OAACV supplementation in this study. No significant effects were observed on the gas emissions of H_2O, methyl mercaptan, and acetic acid among the broiler chickens' treatment groups (Table 4). Chaveerach *et al.* (2004) and Van Immerseel *et al.* (2004) reported that organic acids showed promising results in altering bacterial activities and cecal environment in chicken.

Table 4 – Effects of organic acid supplementation with anti coccidium on broilers' gas emissions[1].

Items, ppm	CON	OA	ACV	OAACV	SEM[2]	*p*-value[3]		
						Anti-coccidium	Organic acid	Interaction
Finish								
NH_3	8.5	8.2	7.2	6.8	1.1	0.448	0.881	0.065
H_2S	2.2	2.9	2.4	2.8	0.5	0.983	0.291	0.752
Methyl mercaptans	1.3	1.2	1.0	1.3	0.2	0.646	0.750	0.504
CO_2	1280	1000	1240	980	92	0.793	0.029	0.930
Acetic acid	1.3	1.2	1.4	1.1	0.2	0.913	0.204	0.665

[1] Abbreviation: CON, (Basal diet with unvaccinated birds); OA, CON + 0.1% organic acid; ACV, CON + anti-coccidium vaccine; OAACV, CON + 0.1% organic acid + anti coccidium vaccine.

[2] Standard error of means.

[3] Means in the same row with different superscripts differ ($p<0.05$). Values represent 11 replicates/treatment, 18 chickens/cage in each of the four treatment groups.

Table 5 – Effects of organic acid supplementation with anti coccidium on broilers' microbial counts[1]

Items, $\log_{10}$cfu/g	CON	OA	ACV	OAACV	SEM[2]	*p*-value[3]		
						Anti-coccidium	Organic acid	Interaction
E. coli	5.05	4.90	5.00	4.98	0.13	0.887	0.528	0.619
Lactobacillus	7.29	7.34	7.31	7.42	0.06	0.325	0.826	0.052
Salmonella	2.61	2.47	2.50	2.51	0.14	0.817	0.620	0.605

[1] Abbreviation: CON, (Basal diet with unvaccinated birds); OA, CON + 0.1% organic acid; ACV, CON + anti-coccidium vaccine; OAACV, CON + 0.1% organic acid + anti coccidium vaccine.

[2] Standard error of means.

[3] Means in the same row with different superscripts differ ($p<0.05$). Values represent 11 replicates/treatment, 18 chickens/cage in each of the four treatment groups.

D'Alessandro & Zolla, (2013) demonstrated that gas emissions are affected by various factors, such as animal husbandry, nutrition, and breeding. However, we believe that the decrease in the feces' ammonia concentration may be due to the increased digestibility and beneficial microflora in broilers' intestines.

According to Liu *et al.* (2020), increased villus height and decreased crypt depth were associated with effective nutrient absorption and better performance. Previously, Samanya & Yamauchi (2002) and Nain *et al.* (2012) demonstrated that diet formulation had modified intestinal morphology, specifically villi height and depth of crypts. Additionally, Baurhoo *et al.* (2007) reported that a healthy gut microbiota improves the turnover of intestinal epithelial cells and decreases inflammation caused by pathogens and toxins. The effect of organic acid supplementation and anti coccidium on small intestinal villus height in broilers is shown in Table 7. Villi length in the duodenum, jejunum, and ileum was unaffected ($p>0.05$) in all treatment groups in the present study. Kettunen *et al.* (2001) reported that the coccidian challenge adversely affects intestine morphology by decreasing the length of villi and decreasing digestive enzyme production (Williams, 2002). The lack of effects on intestinal morphology in inoculated birds could potentially be explained by the small number of vaccinated oocysts. Our results were consistent with other studies (Barreto *et al.*, 2008; Vieira *et al.*, 2008), which also showed that the dietary inclusion of organic acid supplementation had no impact on villus height, crypt depth and intestinal weight. In the present study, the

Table 6 – Effects of organic acid supplementation with anti coccidium on broilers' meat quality[1]

Items	CON	OA	ACV	OAACV	SEM[2]	*p*-value[3]		
						Anti-coccidium	Organic acid	Interaction
pH value	7.75	7.75	7.78	7.75	0.02	0.460	0.404	0.522
Breast muscle color								
Lightness (L*)	63.01	63.34	63.88	62.25	1.59	0.939	0.661	0.512
Redness (a*)	12.48	13.69	12.06	12.27	0.65	0.140	0.246	0.408
Yellowness (b*)	14.21	13.26	14.61	13.56	0.89	0.698	0.273	0.953
WHC, %	59.75	57.86	58.23	58.76	2.59	0.895	0.774	0.610
Cooking loss	13.05	11.93	13.10	12.20	2.13	0.940	0.642	0.958
Drip loss, %								
d 1	7.75	5.06	6.29	6.33	0.84	0.914	0.133	0.124
d 3	12.21	10.78	10.86	11.45	0.75	0.705	0.640	0.262
d 5	17.96	17.59	17.66	15.25	0.93	0.853	0.910	0.067
d 7	24.05	23.44	24.29	22.76	0.76	0.704	0.429	0.072
Relative organ weight, %								
Breast muscle	14.10	14.30	13.93	14.18	0.82	0.870	0.806	0.978
Liver	2.21	2.33	2.16	2.28	0.18	0.760	0.487	0.999
Bursa of Fabricius	0.13	0.12	0.12	0.12	0.02	0.701	0.784	0.956
Abdominal fat	1.82	1.67	1.78	1.51	0.25	0.752	0.528	0.846
Spleen	0.13	0.15	0.12	0.13	0.02	0.789	0.427	0.859
Gizzard	1.63	1.79	1.61	1.58	0.11	0.327	0.553	0.391

[1] Abbreviation: CON, (Basal diet with unvaccinated birds); OA, CON + 0.1% organic acid; ACV, CON + anti-coccidium vaccine; OAACV, CON + 0.1% organic acid + anti coccidium vaccine.

[2] Standard error of means.

[3] Means in the same row with different superscripts differ ($p<0.05$). Values represent 11 replicates/treatment, 18 chickens/cage in each of the four treatment groups.

Table 7 – Effects of organic acid supplementation with anti coccidium on broilers' small intestinal villus height[1]

Items, µm	CON	OA	ACV	OAACV	SEM[2]	*p*-value[3]		
						Anti-coccidium	Organic acid	Interaction
Duodenum	50.34	51.64	49.97	50.64	3.14	0.846	0.781	0.930
Jejunum	44.86	47.35	44.12	45.10	2.28	0.482	0.416	0.721
Ileum	37.11	38.35	36.03	37.40	1.53	0.497	0.387	0.966

[1] Abbreviation: CON, (Basal diet with unvaccinated birds); OA, CON + 0.1% organic acid; ACV, CON + anti-coccidium vaccine; OAACV, CON + 0.1% organic acid + anti coccidium vaccine.

[2] Standard error of means.

[3] Means in the same row with different superscripts differ ($p<0.05$). Values represent 11 replicates/treatment, 18 chickens/cage in each of the four treatment groups.

dietary supplementation of the OAACV group tended to reduced drip loss ($p=0.0675$ and 0.0723) in analyzed meat samples on d5 and d7 (Table 6). However, breast muscle color - Lightness (L*), Redness (a*), and Yellowness (b*) - , organ weight (Breast muscle, Liver, Bursa of Fabricius, Abdominal fat, Spleen, and Gizzard), cooking loss, water holding capacity (WHC), and pH were not affected among the treatments ($p>0.05$). Meat pH is usually a direct expression of the quality of content in muscle acid and influences the sheer intensity, drip loss and color of the meat (Hossain *et al.*, 2015). Kopecký *et al.* (2012) stated that the addition of organic acid supplementation had no significant effect on meat quality in broiler chicken. Also, Islam *et al.* (2008) found that meat quality was not influenced by the inclusion of organic acid supplementation. However, Balasubramanian *et al.* (2018) stated that drip loss was an ordinary indicator of better meat quality. From this perspective, we believe that organic acid supplementation with anti coccidium vaccination treatment has a beneficial effect on meat quality.

CONCLUSION

The present study will be the base of our future research. The dietary inclusion of organic acid supplementation with anti-coccidium vaccine had a beneficial effect on broilers affected by coccidian infections and also improved growth performance, nutrient digestibility, faecal *Lactobacillus* concentration, and reduced NH_3 emission in broilers. Moreover, there were no positive effects observed on intestinal morphology. Therefore, our findings suggested that organic acid supplementation with anti-coccidium vaccine could become a good alternative to improve growth performance and increase beneficial microflora for the poultry industry in the future.

COMPETING INTERESTS

No potential conflict of interest relevant to this article was reported.

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