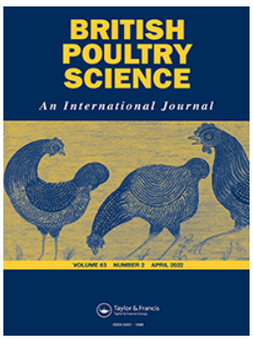

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Performance of broiler chicken on dietary supplementation of protected organic acids blend

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ABSTRACT

1. The fatty acid coated organic acids blend was evaluated for its potential as a growth promoter.
2. A six-week experiment was conducted following a completely randomised design. One-day old broiler chicks (n = 384) were randomly divided into four dietary groups (eight replicates per group). Diet treatments were an unsupplemented basal diet or containing 0.3, 0.6 and 1 g/kg of a coated organic acid blend. Birds were evaluated for growth performance, carcass traits, immune-competence, total viable count and gut villus height.
3. The broiler chickens fed with 1 g/kg organic acids blend showed significantly higher body weight gain with improved feed conversion ratio and lower mortality than those fed the basal diet.
4. The carcass traits *vis*. eviscerated yield, dressing percentage, breast yield and relative weight of giblets, were significantly better in the group fed with 1 g/kg coated organic acids blend with reduction in abdominal fat.
5. Significantly higher cell-mediated, humoral immune responses and villi height with higher lymphoid organ weight (bursa and thymus) and a significant decrease in the total viable count were recorded in birds fed 1 g/kg organic acids blend.
6. The results indicated that dietary inclusion of coated organic acids blend (1 g/kg) improved growth performance, carcass traits, immunity, and gut health in broiler chicken and reduced total viable count and abdominal fat, indicating its potential role as a promising growth promoter in poultry.

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Coated organic acids; performance; broiler; carcass; gut health

Introduction

The growth of the poultry sector is due to improved biogenetics, micronutrient offerings, healthcare and management practices (DAHD, 2019). Specifically designed dietary supplements can boost poultry production and growth performance (Chand et al., 2014; Khan et al., 2014). Among others, acidifiers, such as organic acids (OAs) have been shown to be potentially useful in replacing growth promoters in poultry feed (Partanen and Morz, 1999; Mroz 2005). Since acidification is known to protect against bacteria, fungi and mould, it can be incorporated into preventive nutrition to counter these pathogens (Frank 1994). Studies have demonstrated that many OA, including fumaric acid, formic acid, lactic acid and their salts, can enhance performance and health (Yang et al. 2018). In addition, organic acids can improve feed digestion, nutrient digestibility, and eubiosis in the gut (Ndelekwute et al., 2016).

In animals, OAs play a vital role by reducing the buffering capacity of diet. These acids have a control mechanism to fight harmful microorganisms in the digestive system by lowering the pH in the stomach and the intestine. OA can increase the availability of nutrients in the diet, their assimilation and digestion, and have been shown to improve immune responses in poultry (Yesilbag and Colpan 2006; Abudabos et al., 2014). All these aspects make a significant contribution to the profitability of poultry production. Thus, using an acidifier in livestock nutrition can be a cost-effective performance-enhancing option, exerting its effects through feed, the intestine and in metabolism (Roth et al., 2017). In addition to these advantages, few concerns persist about their palatability, mechanism of action, and neutralisation, allowing scientists to devise alternate ways to utilise them as feed ingredients.

The OAs are rapidly metabolised in the foregut, the crop and the gizzard (Khan and Iqbal, 2016). To counteract this limitation, matrix coating or encapsulation techniques are used for targeted delivery to different gut segments. The dietary matrix coated OAs blend supplementation maintains optimum pH in the intestinal tract and improves nutrient digestibility (Upadhaya et al., 2014a, 2014b).

Besides this, OAs can improve the antibacterial effects of fatty acids (Zentek et al., 2011). The combinations of OA and fatty acids have a beneficial impact on intestinal microecology in piglets (Zentek et al., 2013; Kuang et al., 2015) and nutrient digestibility in laying hens (Lee et al., 2015). Oleic, lauric, palmitic, myristic, and stearic fatty acids are used in feeds to promote plasticising, lubricating, binding, and defoaming properties. In addition, they are used as reagents in the manufacture of feed supplements (Patty 1963; Hawley 1977). Organic acids such as, fumaric, malic and citric acids, are commonly used in poultry industry due to their physical and chemical properties (Dibner and Buttin, 2002). These

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acids have a fruity, tart and sour taste, respectively (Banday et al., 2015; Marques et al., 2020). OA can penetrate bacterial cell walls and disrupt their normal physiology (Dhawale 2005). They reduce the pH of digesta, enhancing pancreatic secretion, and have vital effects on the gastro-intestinal mucosa (Dibner and Buttin, 2002). Moreover, acidification reduces the production of bacterial toxic components and their colonisation of the intestinal wall, preventing damage to epithelial cells (Langhout 2000).

Previous studies have focussed on using unprotected OA or their blends at a dose rate exceeding 1 g/kg of feed (Kim et al., 2015). Studies on protected blends in broiler chickens are very limited, although may be used at lower inclusion rates when compared to unprotected forms. The following study was conducted to understand the effect of blend coated OA at dose of 0.3, 0.6 and 1 g/kg of feed, on broiler growth performance, carcass traits, immunocompetence, total viable bacterial count, and villus height, compared to an antibiotic growth promoter.

Materials and methods

The trial was approved by the Institutional Animal Ethical Committee (Resolution No. XIV/2020- Ref. No. VCU/IAEC/CPCSEA/2020; Dated: 28/08/2020) and the Board of Studies. The experiments were carried out for 6 weeks at the Experimental Broiler Shed and Paraclinical Laboratories of College of Veterinary and Animal Sciences, Udgir.

Broiler management

The experiment was conducted using one-day-old 384 straight-run commercial broiler chicks (Ven Cobb 430Y strain). The one-day-old chicks were equally distributed into four treatment groups randomly. There were eight pen replicates (12 chicks in each pen) for each group; giving 96 broiler chicks per treatment. Following commercial management practices, the birds were reared in a deep litter system using paddy husk as bedding material. Feed and water were made available to the birds *ad libitum*.

Experimental diets

The feed ingredients required for formulating the broiler diet were procured locally. The fatty acid coated blend of stearic acid (57.49%), fumaric acid (17.12%), dl-malic acid (10.22%) and citric acid (15.17%) was procured from Morning Bio Co. Ltd., South Korea. The proximate analysis of the experimental diets was carried out using AOAC (2016) methods. The ingredient and nutrient composition of the experimental diets formulated as per the Bureau of Indian Standards is shown in Table 1. The birds from the control group were fed a standard basal diet containing a commercial antibiotic growth promoter.

The birds from three treatment groups were fed with the basal diet containing 0.3, 0.6, and 1 g/kg blend coated organic acids (BCOA), respectively. All the diets isocaloric and isonitrogenous. The birds were fed a broiler pre-starter (0–7 d of age), broiler starter (8–21 d), and broiler finisher (22–42 d) feed.

Table 1. Calculated composition of experimental broiler diets in different growth phases.

Ingredient (Kgs)	Pre-starter	Starter	Finisher
Maize	54.632	55.62	59.6
Soybean meal	39.205	36.895	31.83
Vegetable Oil	2.49	3.903	4.97
Dicalcium Phosphate (DCP)	1.78	1.83	1.9
Limestone Powder (LSP)	0.89	0.87	0.85
Salt	0.3	0.3	0.3
Trace Min. Premix*	0.11	0.1	0.1
Vitamin premix**	0.15	0.15	0.15
DL-Methionine	0.148	0.131	0.106
L-Lysine	0.13	0.036	0.03
Choline chloride	0.05	0.05	0.05
Toxin binder	0.05	0.05	0.05
Coccidiostat	0.05	0.05	0.05
B-Complex***	0.015	0.015	0.015
Total	100.00	100.00	100.00
Nutrients (%)	Pre starter	Starter	Finisher
Crude protein	23.00	22.00	20.00
Calcium	1.00	1.00	1.00
Available Phosphorus	0.45	0.45	0.45
L-Lysine	1.34	1.20	1.06
DL-Methionine	0.53	0.50	0.45
ME, kcal/kg	3000.91	3099.17	3200.67

*Trace mineral premix supplied Mg- 300, Mn- 55, I- 0.4, Fe- 56, Zn-30 and Cu- 4 mg/kg diet.

**The vitamin premixes supplied Retinol 2475 mcg, Cholecalciferol 30 mcg; Menaquinone 1 mg; Tocopherol 26.8 mg d-alpha tocopherol /kg diet.

***B-complex supplied Thiamine 2 mg, Riboflavin 4 mg, Cyanocobalamin 10mcg; Niacin 60 mg; Pantothenic acid 10 mg/kg diet

Performance study

The birds were weighed weekly individually to record their body weights. Weightgain, feed intake, cumulative weekly feed intake and cumulative weekly feed conversion ratio were calculated for each treatment group. The feed intake was recorded by offering the weighed quantity of feed and subtracting the left-over residue. The mortality, if any, was recorded and expressed in percentage.

Immune response

The cell-mediated (CMI) and humoral (AMI) immune responses of broiler chicks were assessed using the *in vivo* foot web index (cutaneous basophilic hypersensitivity test) to phytohemagglutinin (a lectin from *Phaseolus vulgaris*-PHAP) and serum antibody titres to *Newcastle disease virus* (NDV), respectively.

The foot web index to PHA-P was calculated as per Corrier and Deloach (1990). On the 22nd day post-hatch, 16 birds from each treatment were randomly selected, and the toe thickness of both left and right foot at 3rd and 4th interdigital spaces were measured by a digital micrometer. Immediately after measurements, 0.1 ml PHA-P (1 mg/ml) was intradermally injected into the right foot web while 0.1 ml of phosphate buffer saline (PBS) was injected into the left foot web as a placebo. The web swellings of both feet were measured after 24 h. The *in vivo* response to PHA-P was expressed as a web index.

To measure the humoral immune response, the broilers were vaccinated with LaSota vaccine on the 5th day of age and booster on d 21. The humoral immune response was determined by estimating serum antibody titres against NDV. Blood was collected from two birds per replicate and 16 birds per treatment group. The blood was allowed to clot at room temperature and centrifuged at 1500 rpm for 10 min. The clear upper serum layer was carefully extracted and stored at −20°C

until analysis. A haemagglutination inhibition (HI) test was performed in a U bottom microtitre plate using 4 HA unit ND antigen. The reciprocal of the highest dilution of serum showing 50% HI (button formation) was taken as the HI titre (log 2).

The weight of immune organs (bursa of Fabricius, thymus, and spleen) was measured using digital weighing balance at the end of trial by slaughtering one bird per replicate and was expressed in percentage of live weight.

Carcass traits

At the end of the experiment, eight birds with body weights closest to the mean of each pen were selected. The birds deprived of feed for 8 h before slaughter, but clean, potable drinking water was available. Measured parameters included de-feathered weight, dressed weight, eviscerated weight, cut-up parts (breast, thigh, drumsticks, back, neck and wing) weight, giblet (heart, liver, and gizzard) weight, and abdominal fat pad thickness.

Gut health

The total viable counts from gut digesta were measured on d 42 using eight slaughtered birds per treatment group (i.e. one bird per replicate). The caecal contents (1 g) were collected in a sterile glass tube under aseptic conditions and serially diluted in sterile normal saline to obtain 10^{-5}, 10^{-6}, and 10^{-7} dilutions to obtain total viable counts of bacteria (TVC). From each dilution, 1 ml was mixed with 9 ml of molten brain heart infusion agar (50°C) in a petri dish. After solidification, these were incubated at 37°C in a bacteriological incubator for 24 h. All bacterial growth was counted as colony forming units (CFU). The total viable count (CFU/g) was determined as a product of the mean of colonies at particular dilution and dilution factor.

Tissue samples of approximately 1 cm in size were collected from the midpoint of duodenum, jejunum, and ileum and fixed in 10% neutral buffered formalin. The fixed tissues were dehydrated by immersing in ethyl alcohol of increasing concentrations (from 50% to absolute), cleared in xylene and embedded in paraffin. Paraffin sections of 3–5 µm thickness were cut with a rotary microtome and stained using the haematoxylin and eosin staining technique. Villus height (from the duodenum, jejunum, and ileum) was measured from the tip of the villi to the crypt mouth under a light microscope (Olympus BX53) using Imaging Software (Cell Sens Standard, Olympus Corporation, Japan) and an Olympus DP73 camera.

Statistical analysis

Data were analysed as a completely randomised design in a one-way ANOVA (Snedecor and Cochran 1994) to test the effect of increasing levels of BCOA supplementation. Microbial counts were expressed as log10-transformed data for total colony forming units (CFU). All analysis of data was conducted using SPSS software package version 20.0. Variables having unequal observations were analysed following the least square design method and Duncan's multiple range test (Duncan, 1955). Results were considered significant at 95% confidence limits ($P < 0.05$).

Results

Growth performance

The results for body weight gain, feed intake, and FCR are shown in Table 2. The 42 d body weight gain for birds fed the control diet was 647.70, whereas those fed the diets containing 0.3, 0.6, and 1 g/kg BCOA, were 665.8, 661.9, and 700.6 grams, respectively. The overall body weight gains (g/bird) at 42 d for the control, and 0.3, 0.6, and 1 g/kg BCOA were 2653.4, 2713.5, 2740.0, and 2806.7, respectively. Weight gain was significantly higher with better FCR in birds fed 1 g/kg BCOA followed by 0.6 g/kg and 0.3 g/kg compared to the birds fed the basal diet alone. The dietary inclusion of BCOA had a significant ($P < 0.001$) effect on body weight gain during the starter (0–3 weeks), finisher (3–6 weeks) and overall (0–6 week) growth phases. The birds receiving 1 g/kg BCOA had higher body weight gain ($P < 0.001$) during starter (0–3 weeks), finisher (3–6 weeks), and overall (0–6 weeks) growth phase.

In birds fed BCOA, non-significant improvements in FCR were recorded during the first 2 weeks of the trial, while better ($P < 0.001$) FCR was observed from 21 d onwards. Overall FCR was 1.56, 1.54, 1.53 and 1.50 for the control, 0.3, 0.6, and 1 g/kg BCOA treatment groups, respectively. Among the treatment groups, improved FCR was recorded in birds fed 1 g/kg BCOA.

Carcass traits

The data for carcass traits, relative weight of visceral organs, cut-up parts yield and abdominal fat pad thickness of the broilers are given in Table 3. Shrinkage and feather loss were comparable among various dietary treatment groups. Blood loss was significantly ($P < 0.05$) higher when birds were supplemented with 1 g/kg BCOA.

Table 2. Effect of dietary addition of blend coated OAs on broiler body weight gain, feed intake and feed conversion ratio.

Age/ Phase	Parameter	Control	Blend coated OAs (g/kg) 0.3	0.6	1.0	Pooled SEM	*P* value
I wk	BWG	142.3^{a}	144.5^{b}	146.8^{c}	149.6^{d}	0.352	0.000
	FI	141	142	143	142	0.930	0.964
	FCR	0.99	0.98	0.97	0.95	0.007	0.042
II wk	BWG	303.8^{a}	307.0^{ab}	308.1^{ab}	310.8^{b}	0.670	0.021
	FI	326	324	323	319	2.560	0.942
	FCR	1.07	1.06	1.05	1.03	0.009	0.546
III wk	BWG	488.0^{a}	491.9^{a}	500.5^{b}	503.0^{b}	1.382	0.001
	FI	656	651	657	643	2.020	0.218
	FCR	1.34^{c}	1.32^{b}	1.31^{b}	1.28^{a}	0.004	0.000
IV wk	BWG	528.0^{a}	542.9^{ab}	544.3^{ab}	550.7^{b}	2.688	0.05
	FI	805	817	810	805	4.180	0.878
	FCR	1.52^{d}	1.50^{bc}	1.49^{b}	1.46^{a}	0.004	0.000
V wk	BWG	543.8^{a}	561.5^{ab}	578.6^{bc}	599.4^{c}	4.135	0.000
	FI	928	936	953	969	9.140	0.630
	FCR	1.70^{d}	1.67^{bc}	1.65^{b}	1.62^{a}	0.006	0.000
VI wk	BWG	647.7^{a}	665.8^{ab}	661.9^{ab}	700.6^{b}	6.701	0.05
	FI	1283	1300	1286	1334	13.570	0.767
	FCR	1.98^{d}	1.95^{bc}	1.94^{b}	1.90^{a}	0.005	0.000
0-III wk	BWG	933.9^{a}	943.2^{b}	955.1^{c}	963.4^{d}	1.351	0.000
	FI	1122	1118	1123	1104	4.100	0.633
	FCR	1.22^{b}	1.19^{ab}	1.19^{ab}	1.15^{a}	0.008	0.05
III–VI wk	BWG	1719.5^{a}	1770.3^{b}	1784.8^{b}	1843.3^{c}	6.767	0.000
	FI	3015	3053	3048	3108	13.790	0.242
	FCR	1.75^{d}	1.72^{c}	1.71^{b}	1.68^{a}	0.004	0.000
0-VI wk	BWG	2653.4^{a}	2713.5^{bc}	2740.0^{c}	2806.7^{d}	7.141	0.000
	FI	4137	4171	4171	4213	13.970	0.450
	FCR	1.56^{c}	1.54^{bc}	1.53^{b}	1.50^{a}	0.005	0.000

Values bearing different superscript differed significantly; NS = Non-significant ($P > 0.05$)

Table 3. Effect of dietary addition of blend coated OAs on broiler carcass traits.

Traits	Control	Blend coated OAs (g/kg) 0.3	0.6	1.0	Pooled SEM	*P* value
Shrinkage	4.77	4.75	4.72	4.66	0.031	0.838
Blood loss	2.54^{a}	2.54^{a}	2.56^{ab}	2.58^{b}	0.005	0.006
Feather loss	5.31	5.29	5.28	5.29	0.007	0.727
Eviscerated yield	69.18^{a}	69.62^{abc}	69.91^{bc}	70.17^{c}	0.109	0.029
Carcass yield	74.23^{a}	74.82^{abc}	75.15^{bc}	75.49^{c}	0.121	0.004
Heart	0.77^{a}	0.79^{ab}	0.80^{ab}	0.81^{b}	0.005	0.046
Liver	2.21^{a}	2.30^{bc}	2.32^{c}	2.35^{c}	0.012	0.001
Gizzard	2.06^{a}	2.11^{ab}	2.13^{ab}	2.16^{b}	0.011	0.026
Giblet	5.05^{a}	5.20^{bc}	5.24^{c}	5.32^{c}	0.023	0.000
Breast	20.85^{a}	21.10^{abc}	21.20^{bc}	21.28^{c}	0.045	0.012
Back	16.43	16.44	16.49	16.52	0.035	0.904
Drumstick	10.48^{a}	10.55^{ab}	10.62^{b}	10.65^{b}	0.019	0.028
Thigh	10.21	10.28	10.32	10.35	0.023	0.349
Neck	3.58^{a}	3.59^{a}	3.61^{a}	3.67^{b}	0.010	0.004
Wing	7.64	7.68	7.67	7.70	0.030	0.959
Abdominal Fat pad Thickness	0.54^{c}	0.50^{b}	0.50^{b}	0.45^{a}	0.006	0.000

Values bearing different superscript differed significantly; NS = Non-significant ($P > 0.05$)

A significant ($P < 0.05$) improvement in eviscerated and dressed yield was observed in birds fed BCOA. Dressing percentage for the control, 0.3, 0.6, and 1 g/kg BCOA was 74.23%, 74.82%, 75.15% and 75.49%, respectively. A significant ($P < 0.05$) effect on the relative yield of heart, liver, gizzard and giblets was observed due to the dietary addition of BCOA. Among cut-up parts yield, the relative weight of the back, thigh and wing were comparable. A significant ($P < 0.012$) improvement in breast yield was observed in BCOA-fed birds. The breast yield in the control, 0.3, 0.6 and 1 g/kg of BCOA treatment groups was 20.85%, 21.10%, 21.20% and 21.28%, respectively. Dietary addition of 1 g/kg BCOA significantly reduced-fat pad thickness ($P < 0.001$) compared to other dietary treatments.

Immunity and total viable count

The data on immune responses and total viable count (TVC) are presented in Table 4. A significant ($P < 0.05$) increase in CMI and AMI was observed in birds fed BCOA. The mean of log_2 serum antibody titres in the control, 0.3, 0.6 and 1 g/kg BCOA treatment groups were 4.25, 6.38, 6.31 and 6.81, respectively. Relative spleen weight did not differ significantly amongst treatment groups. However, significantly ($P < 0.05$) higher bursal and thymus weights were recorded from 1 g/kg BCOA-fed birds. At the end of the trial, TVC were significantly ($P < 0.001$) different among dietary treatments. The birds receiving 0.6 or 1 g/kg BCOA lowered TVC compared to samples from the control birds. However, dietary addition of 0.3 g/kg BCOA showed significantly high TVC.

Height of intestinal villi

The data on intestinal villi height of duodenum, jejunum, and ileum at 42 d are shown in Table 4 and Figure 1. Villus height was significantly ($P < 0.001$) different among dietary treatments. The birds receiving 0.6 or 1 g/kg BCOA had greater villus height in samples from the duodenum, jejunum, and ileum compared to birds from the control groups. Dietary addition of 1 g/kg BCOA had the greatest villi height

Table 4. Effect of dietary addition of blend coated OAs on broiler immunocompetence and digesta total viable count and gut villi height.

	Control	Blend coated OAs (g/kg) 0.3	0.6	1.0	Pooled SEM	*P* value
CMI PHA-P foot web index (mm)	0.22^{a}	0.26^{b}	0.26^{b}	0.27^{b}	0.005	0.005
Humoral-HI titer against ND (log2)	4.25^{a}	6.38^{b}	6.31^{b}	6.81^{b}	0.158	0.000
Bursa (% of live wt.)	0.138^{a}	0.146^{ab}	0.155^{bc}	0.165^{c}	0.003	0.017
Spleen (% of live wt.)	0.211	0.215	0.221	0.220	0.002	0.431
Thymus (% of live wt.)	0.398^{a}	0.419^{b}	0.429^{bc}	0.436^{c}	0.003	0.000
TVC CFU/gm (log10)	0.461 x10^{10c}	0.492 x10^{10d}	0.146 x10^{10a}	0.138 x10^{10a}	0.026	0.000
Duodenum (µm)	1096.15^{a}	1282.50^{b}	1299.11^{b}	1405.47^{c}	20.360	0.000
Jejunum (µm)	1016.38^{a}	1026.79^{a}	1102.68^{b}	1108.40^{b}	9.110	0.001
Ileum (µm)	811.30^{a}	831.30^{a}	986.92^{b}	1014.01^{b}	12.780	0.000

Values bearing different superscript differed significantly; NS = Non-significant ($P > 0.05$)

compared to other dietary treatments. The birds fed the diet containing 0.3 g/kg BCOA had no significant effect on villus height of jejunum and ileum, but showed a signified increase in villus height in the duodenum compared to birds fed the control diet.

Discussion

The present study's findings showed that feeding supplemental BCOA to broilers enhanced growth performance, immunity and gut health. The birds fed the BCOA showed increased body weight gain throughout the experiment. This weight gain might be due to improvement in the digestion and absorption of the essential nutrients in the gastrointestinal tract. The reduction in intestinal pH due to organic acids favoured the growth of beneficial microbes suppressing the pathogenic bacteria (Mroz et al., 2006). The decrease in the bacterial count in the intestines curtails the microbial competition for host nutrients and ensures the availability of more nutrients for the beneficial microbes and the host. Further, the BCOA improved intestinal morphology, which might have enhanced the digestive enzyme activities and, thereby, the nutrient availability and uptake. Supplementation with BCOA (1 g/kg) caused significant improvement in growth performance than the birds fed with only basal diet. Earlier reports stated positive effects of dietary supplementation of OA on broiler performance (Leeson et al., 2005; Fascina et al., 2012 and Islam et al., 2018). However, Nguyen et al. (2018) and Salah et al. (2019) reported a non-significant effect on body weight gain after supplementation with a blend of OA.

Though the present findings showed a non-significant increase in overall feed consumption after dietary inclusion of BCOA, a significant improvement was recorded in the overall feed conversion ratio compared to the birds fed the basal diet. The numerical increase in the overall feed intake

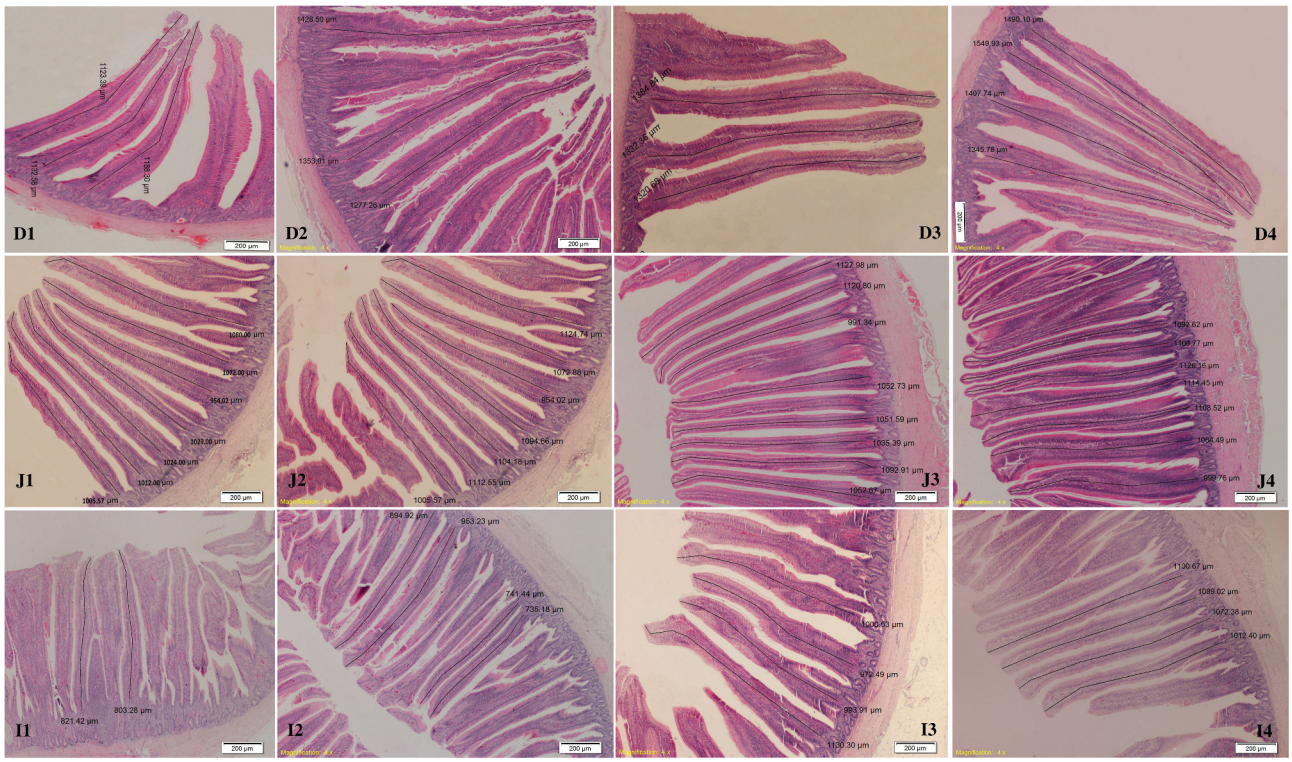

Figure 1. Effect of dietary addition of blend coated OAs on Villi hights of Small Intestines (Duodenum - D1: Control, D2: 0.03%, D3: 0.06% and D4: 0.1% blend coated OAs; Jejunum - J1: Control, J2: 0.03%, J3: 0.06% and J4: 0.1% blend coated OAs; Ileum - I1: Control, I2: 0.03%, I3: 0.06% and I4: 0.1% blend coated OAs).

suggested improved gut health, absorption and bioavailability of nutrients, which resulted in the better feed conversion ratio. Supplementation with acidifiers in the feed is known to maintain an acidic pH in the proventriculus and gizzard for optimum enzymatic activities. Blend coating of OA ensures their delivery at the site of action, i.e. in the small intestines, which promotes absorption of nutrients. The present findings for feed intake were in agreement with earlier work (Nguyen et al., 2018), where it was reported that the dietary addition of BCOA had no significant effect on weekly and overall feed intake. Similar findings have been reported by Rodriguez-Lecompte et al. (2012); Sarvari et al. (2015); Basmacioglu-Malayoglu et al. (2016); Ndelekwute et al. (2016); Pathak et al. (2017) and Khatibjoo et al. (2018) with better FCR (Sarvari et al., 2015).

In the present study, mortality remained within normal commercial levels in all treatment groups, however, the birds supplemented with BCOA had less mortality than the control group. Similar results were documented earlier in feeding trial with acidifiers (Brzoska et al., 2013; Youssef et al., 2017).

Eviscerated yield, dressed yield, breast yield, and relative yield of giblet were significantly improved and abdominal fat pad thickness was significantly decreased, which was in agreement with previous trials where the birds fed 0.2% butyrate (Leeson et al., 2005), a mixture of fumaric acid, calcium formate, calcium propionate, potassium sorbate, and hydrogenated vegetable oil (Hassan et al., 2010), and OAs (Izat et al., 1990; Khan et al., 2016; Heidari et al., 2018; Salah et al., 2019). At present, no published trials have evaluated the role of BCOA on reducing back fat thickness. The effect of BCOA on intestinal morphology, digestive enzyme activities and the growth of beneficial microbes might have enhanced the carcass traits, due to improved gut health, digestion and absorption of nutrients in the small intestines.

A significant increase in the relative weight of bursa and thymus indicated that the addition of blend coated OAs resulted in better immune responses compared to the control birds, as evidenced by humoral and cell-mediated immune responses. The increase in growth performance was associated with a significant increase in the cell-mediated and humoral immune response along with relative weights of the bursa and thymus. This indicated better physiological status and immune response of the broilers throughout the experimental period. Earlier, Abdel-Fattah et al. (2008), Hassan et al. (2010), and Lee et al. (2017) reported increased relative weights of the immune organs and improvement in immune functions in the birds fed with citric acid and OAs, respectively. The OAs supplementation can potentiate the regulatory T cells with a higher percentage of CD4+, CD25 + and T-cell (Lee et al., 2017).

In the present study, dietary addition of 1 g/kg blend coated OAs significantly reduced the caecal bacterial load. The decrease in the total viable count may have been due to the antibacterial activity of the BCOA, which diffuse into the bacterial cells in an undissociated form, resulting in the reduction of intracellular pH, suppression of nutrient transport system, destruction of cytoplasmatic enzymes and detachment of ATP-driven pumps, leading to death (Hsiao and Siebert, 1999). In contrast, Ozturk et al. (2004) observed that the addition of OAs had no significant effect on the bacterial load of the gastro-intestinal tract. Lower pH in the feed and digestive tract due to acidifiers exerts antimicrobial activity in poultry (Desai et al., 2007). Lower intestinal pH suppresses growth of pathogenic bacteria which reduces competition for nutrients. Pathogenic bacteria, such as *E. coli, Salmonella* spp., and *Clostridium perfringens*, are then eliminated (Van-Immerseel et al., 2004a, 2004b), while acid-tolerant beneficial bacteria like *Lactobacillus* and *Bifidobacterium* spp. survive under such conditions (Mroz et al., 2006). Such modulation in gut

microflora benefits the host metabolism (Dibner and Buttin, 2002a, 2002b), improving host performance. The findings of the present study are in line with the work of Alshawabkeh and Tabbaa (2002). McEwen and Fedorka Cray (2002) demonstrated the antibacterial activity of OA whereby supplementation reduced bacterial colonisation in the gastrointestinal tract. Cherrington et al. (1991) shifted the microbiome towards Gram-positive microflora. Additionally, suppression of Gram-negative bacteria due to supplementation of OA can create a favourable environment for the growth of *Lactobacilli* spp. owing to reduced competition for host nutrients (Garrido et al., 2004).

The results for villus height were in line with earlier work by Xia et al. (2004) and Adil et al. (2010), who reported that dietary addition of OA resulted in higher villus height in the duodenum and jejunum. Leeson et al. (2005) observed that dietary addition of 0.4% butyrate glycerides increased villus height. They stated that butyrate helped to maintain villi structure.

In the present study, the increase in villus height in different segments of the small intestine could be ascribed to the important part of the gut epithelium as a barrier against harmful metabolites from pathogenic bacteria in the lumen of the intestine. The salts of OA diminish the growth of pathogenic and non-pathogenic bacteria present in the intestine. This can result in a reduction of intestinal colonisation and infectious progression, reducing any inflammation in the mucosa of the intestine, increasing villus height, enhancing secretion and thereby improving digestion and absorption of the nutrients (Iji and Tivey, 1998). The improved performance seen in the broilers in the current trial may have been because of an increase in the height of the villus, which would afford a greater surface area for the absorption of nutrients. To achieve better growth and feed efficiency, the health of the intestine has great importance. During the action of antimicrobials, the morphology of intestinal structure might change, resulting in shorter villi and deeper crypts (Xu et al., 2003).

In conclusion, dietary inclusion of BCOA (1 g/kg) improved broiler growth performance, carcass traits, immunity, gut health and liveability compared to birds fed the basal control diet. However, the effects of BCOA need to be explored in other poultry species and agroclimatic conditions, taking a number of other parameters into consideration.

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