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Original Article

Influence of Feed Additive Supplementation on Growth Response, Gut Health, and Meat Quality of Broiler Chicken

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ABSTRACT

The effects of organic acid or synbiotic and their mixture on the growth performance, gut morphology, intestinal microbiota, and meat quality of broiler chicken were investigated in a 42-day feeding trial. One hundred and forty (140) one-day-old broiler chicks were randomly allocated into 4 diets with 5 replicates of 7 birds each. Diet 1 was a basal diet (control diet). Diets 2, 3 and 4 contained the basal diet +0.1% organic acid, basal diet+0.05% synbiotic, basal diet + 0.1% organic acid + 0.05% synbiotic respectively. Performance indices were measured. On day 42, ileal digesta samples were collected from three birds per replicate for microbial counts while about 5 cm of distal ileum was severed for morphological measurements. Also, meats were sampled for microbial count, physicochemical assessment, and thiobarbituric acid reactive substances (TBARS). Data were analyzed using descriptive statistics and ANOVA at α0.05. At the starter phase, lower feed conversion ratio (1.84) was recorded in birds fed blend of organic acid + synbiotic diet. At the finisher phase, improved FCR (1.38) was observed in birds that received organic acid diet, though, similar to the FCR of birds fed synbiotic diet (1.64). Lowest *Escherichia coli* counts (0.57×10^5 CFU/mL) (P= 0.025) were recorded in birds fed the blend diet. Increased lactic acid bacteria

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counts ($P < 0.0001$) were observed in birds fed organic acid diet. Crypt depth (161.78 µm) and villus height: crypt depth ratio (4.48) of birds fed the control diet were similar to those on the other diets. Breast meat of birds fed organic acid diet (26.22%) was significantly bigger (22.93%) than the control diet. The crude protein of birds fed organic acid diet (22.96%) and synbiotic (22.87%) were similar but the blend (24.83%) was significantly ($P<0.05$) higher than the basal diet (21.92%). The lowest water holding capacity (64.67%) was recorded in birds fed the basal diet while highest values (70.67%) were found in the synbiotic and blend diets. TBARs of broiler chicken meat in birds fed organic acid (0.13 mgMDA/100g), synbiotic (0.12 mgMDA/100g) and the blend (0.14 mgMDA/100g) were similar but lower than the control diet (0.22 mgMDA/100g). The microbial counts of the meat show that the total Lactobacillus count of birds on synbiotic diet (0.58×10^4CFU/g) and the combination of synbiotic plus organic acid (0.65×10^4 CFU/g) were similar but significantly ($P<0.05$) higher than the control diet (0.34×10^4 CFU/g) and organic acid diet (0.20×10^4 CFU/g). Salmonella count was lowest in the blend (0.32×10^3 CFU/g) and highest in organic acid diet (0.86×10^3 CFU/g) In conclusion, dietary supplementation of 1g/kg organic acid singly or plus 0.5g/kg synbiotic improved the feed conversion ratio, gut health, and meat quality of broiler chicken and can conveniently replace antibiotic growth promoters in broilers nutrition.

Keywords: Acidifier, Broiler Chicks, Meat Quality, Microbiome, Morphology, Synbiotic

INTRODUCTION

The Administration of antibiotics and antimicrobials at sub-therapeutic level is widely practiced to regulate infectious microbes and to enhance productivity in food animals (Cogliani *et al*., 2011). However, indiscriminate use of antibiotics may affect humans directly through residues of antibiotics in meat or indirectly through transference of resistance genes from animal to human microbiota or through the selection of antibiotic resistance determinants that may spread to the human pathogen (Aminov and Mackie, 2007). As a result of this, a ban was issued by European Union (EU, 2006) and National Agency for Food and Drug Administration and Control (NAFDAC, 2018) against the use of antibiotic growth promoters (AGP) in animal feed. Therefore, it becomes imperative to explore natural feed additives that can conveniently replace AGP in poultry production.

The use of natural promoters such as prebiotics, probiotics, synbiotics, phytogenics, enzymes, oligosaccharides, essential oils, clay minerals, immunostimulants, toxic binders, organic acids, and other feed additives, to enhance gut health and growth performance of broiler chickens have been advocated (Agboola and Omidiwura, 2020; Borazjanizadeh *et al*., 2011).

Acidifiers/organic acids possess the ability to modulate the pH of feed as well as that of the alimentary canal and with this ability, they can inhibit the growth of pathogenic bacteria (Arogbodo *et al*., 2020). They have also been reported to prevent damage to epithelial cells and reduce the production of toxic components by the bacteria and colonization of pathogens on the intestinal wall (Langhout, 2000).

Synbiotics, a combination of probiotics and prebiotics which affect the host by improving the survival and implantation of live microbial dietary supplements in the gastrointestinal tract (Awad *et al*., 2009). It selectively stimulates the growth and/ or activates the metabolism of one or limited number of health-promoting bacteria (Rahul Sharma *et al*., 2018).

In terms of consumers' preferences, broiler meat should have good nutritional characteristics (Mir *et al*., 2017) as well as good keeping qualities. The safety quality of meat can be estimated using total viable counts however, this does not necessarily determine its sanitary quality rather it is the range of its different groups of microorganisms which determines if the product is safe (Jay, 1992).

Based on this background, the study aimed to investigate the effects of diets supplemented with an organic acid, synbiotic, or their combination on growth response, gut morphology, intestinal microbiota, and meat quality of broiler chickens.

MATERIAL AND METHODS

Experimental site

The experiment was carried out at the Poultry Unit of the Teaching and Research Farm, University of Ibadan, Oyo State, Nigeria. This study complied with the University of Ibadan ethics requirements for animal handling.

Experimental diets and management of birds

One-hundred and forty (140) one-day-old Arbor acre broiler chicks were obtained from a reputable hatchery for this study. The birds were weighed, tagged, and randomly allotted to four dietary treatments with five replicates of seven birds per replicate in a completely randomized design. Treatment 1 consisted the basal diet (control diet): Treatment 2: basal diet + 0.1% organic acid, Treatment 3 was basal diet + 0.05% synbiotic and Treatment 4: basal diet + 0.1% organic acid + 0.05% synbiotic. Experimental diets for starter phase (Table 1) and finisher phase (Table 2) were formulated to meet the nutrient requirements of broiler chicken according to the NRC (1994) recommendation. Two commercial feed additives: synbiotic (Bacflora-F®) and organic acid (Turbotox®) were used in this study. Bacflora® contains a probiotic and a prebiotic of *B. licheniformis, B. subtilis, E. faecium, L. acidiphilus, S. cerevisiae,* and Citric Acid, Orthosphosphoric Acid, Lactic Acid, Calcium Carbonate, Magnesium Oxide. Turbotox® consists of a mixture of Formic acid, Citric acid, and Propionic acid. The additives were products of Xvet Company, Germany. The experimental diets and water were supplied *ad libitum* throughout the study period that lasted for 42 days. Also, proper vaccination and routine management were strictly adhered to.

Table 1: Gross composition (g/kg) of experimental diets for broiler chicken (0-21 days)

Ingredient	basal diet (control diet)	basal+organic acid	basal + synbiotic	basal + synbiotic + organic acid
Maize	520.00	520.00	520.00	520.00
Soybean meal	410.00	409.00	409.50	408.50
Fish meal	10.00	10.00	10.00	10.00
Soya oil	20.50	20.5	20.50	20.50
Limestone	10.00	10.00	10.00	10.00
DCP	20.00	20.00	20.00	20.00
Broiler premixes	3.00	3.00	3.00	3.00
Lysine	1.00	1.00	1.00	1.00
Methionine	2.50	2.50	2.50	2.50
Table Salt	3.00	3.00	3.00	3.00
Organic acid	0.00	1.00	0.00	1.00
Synbiotic	0.00	0.00	0.50	0.50
Total	1000	1000	1000	1000.
Calculated nutrients				
Crude Protein(g/kg)	231.2	231.2	231.2	231.2
ME (Kcal/kgDM)	3011	3011	3011	3011
Ether Extract (g/kg)	40.1	40.1	40.1	40.1
Crude Fibre (g/kg)	38.2	38.2	38.2	38.2
Calcium g/kg	9.7	9.7	9.7	9.7
Total P (g/kg)	7.7	7.7	7.7	7.7
NonPhytate P g/kg	3.9	3.9	3.9	3.9
Ca:NPP	2.50	2.50	2.50	2.50
Ca:total P	1.26	01.26	1.26	1.26

DCP- Dicalcium phosphate, ME- Metabolizable Energy, NPP-Non phytate Phosphorus, Ca - Calcium
Composition of Premix per Kg of diet: vitamin A, 12,500 I.U; vitamin D3, 2,500 I.U; vitamin E, 40mg; vitamin K3, 2mg; vitamin B1, 3mg; vitamin B2, 5.5mg; niacin, 55mg; calcium pantothenate, 11.5mg; vitamin B6, 5mg; vitamin B12, 0.025mg; choline chloride, 500mg; folic acid, 1mg; biotin, 0.08mg; manganese, 120mg; iron, 100mg; zinc, 80mg; copper, 8.5mg; iodine, 1.5mg; cobalt, 0.3mg; selenium, 0.12mg; Anti-oxidant, 120mg

Table 2: Gross composition (g/kg) of experimental diets for broiler chicken (22-42 days)

Ingredient	basal diet (control diet)	basal+organic acid	basal + synbiotic	basal + synbiotic + organic acid
Maize	560.00	560.00	560.00	560.00
Soybean meal	360.00	359.00	359.50	358.50
Soya oil	30.00	30.00	30.00	30.00
Bone meal	14.00	14.00	14.00	14.00
Limestone	13.00	13.00	13.0	13.00
DCP	13.00	13.00	13.00	13.00
Broiler premixes	2.50	2.50	2.50	2.50
Lysine	2.50	2.50	2.50	2.50
Methionine	2.50	2.50	2.50	2.50
Organic acid	0.00	1.00	0.00	1.00
Synbiotic	0.00	0.00	0.50	0.50
Table Salt	2.50	2.50	2.50	2.5
Total	1000	1000	1000	1000
Calculated nutrients				
Crude Protein g/kg	207.2	207.2	207.2	207.2
ME (Kcal/kgDM)	3074	3074	3074	3074
Ether Extract g/kg	38.6	38.6	38.6	38.6
Crude Fibre g/kg	35.7	35.7	35.7	35.7
Calcium g/kg	13.4	13.4	13.4	13.4
Total P g/kg	8.00	8.00	8.00	80.0
NonPhytate P g/kg	2.6	2.6	2.6	2.6
Ca:NPP	5.23	5.23	5.23	5.23
Ca:total P	1.69	1.69	1.69	1.69

DCP- Dicalcium phosphate, ME- Metabolizable Energy, NPP-Non phytate Phosphorus, Ca - Calcium
Composition of Premix per Kg of diet: vitamin A, 12,500 I.U; vitamin D3, 2,500 I.U; vitamin E, 40mg; vitamin K3, 2mg; vitamin B1, 3mg; vitamin B2, 5.5mg; niacin, 55mg; calcium pantothenate, 11.5mg; vitamin B6, 5mg; vitamin B12, 0.025mg; choline chloride, 500mg; folic acid, 1mg; biotin, 0.08mg; manganese, 120mg; iron, 100mg; zinc, 80mg; copper, 8.5mg; iodine, 1.5mg; cobalt, 0.3mg; selenium, 0.12mg; Anti-oxidant, 120mg

Data and sample collection

Growth performance

Feed Intake (FI): This was the measurement of quantity of feed consumed weekly. FI= Amount of feed consumed –Amount of feed remaining.

Body weight gain: This was estimated by subtracting the initial weight from the final weight gain at the end of starter and finisher phases.

$$\text{Feed Conversion Ratio} = \frac{\text{Average feed intake (g)}}{\text{Average body weight gain (g)}}$$

Gut morphology

On day 42, three birds were selected from each treatment group, weighed, and slaughtered. The Ileal samples were collected from the two-third section between Meckel's diverticulum and ileo-caeco-colonic junction. Ileal samples were fixed in 10% phosphate-buffered formalin for a minimum of 48 h, and 4.0-µm sections were prepared. The sections were stained with standard hematoxylin-eosin solution and observed for villus height, villus width, crypt depth, epithelial thickness at 100X magnification by light microscopy using a calibrated ocular micrometer.

Ileal microbial count

Digesta samples were collected from the ileum of each bird for microbial count and stored in a sterile container and refrigerated at 4ºC. The culture media were prepared 24 hours before collected samples were poured into petri-dishes. To examine the count of Lactobacilli (Man Rogosa Sharpe agar, incubated anaerobically 48h); Total bacteria count (nutrient agar, incubated aerobically 24h)*; Escherichia coli* (Eosin methyl blue agar and Salmonella (*Salmonella shigella* Agar, incubated aerobically 24h) were used. One milliliter of the digesta was added to a 9ml pre-reduced salt medium in other tubes. The suspension was prepared from 10^{-1} dilution and serial dilutions were done (10^{-2} to 10^{-5}), then serial dilution at10^{-3} and 10^{-5} was used to culture the media. From the dilution, 0.1 ml of the sample was plated onto the appropriate medium for enumeration of bacteria. Discrete colonies on plates were counted using a colony counter and count were estimated in logarithm number of bacteria per 1g sample (log10 CFU/g).

Meat quality assessment

Two birds per replicate were sacrificed. The birds were weighed before and after exsanguination, then defeathered and weighed. Each carcass was cut into primal cuts and each cut was weighed to determine carcass characteristics. The organs were harvested and also weighed using a sensitive scale.

Proximate analysis

Proximate analysis of the experimental diets and raw meat samples was done to determine the moisture content, crude fiber, ash content, crude protein, and ether extract according to the methods described by AOAC (2009).

Water holding capacity

This was determined following the procedure of Dzudie *et al.,* (2005) with some modifications. A sample of 0.3 g was placed between two filter paper grade 1 and pressed between two 12x12 cm plexi-glass plates for 20 min under 1 kg of weight pressure. Due to the force exerted on the sample, the released liquids were impregnated in the paper and they were considered meat-free water. In addition, pressed meat area and liquid released, were determined following the Image J software (Image j® 1.40 g, Wayne Rasband, National Institutes of Health, USA). Water holding capacity was performed in triplicate and was determined using the following equation

$$\text{Water release} = \frac{(\text{total surface area} - \text{meat layer area})\ \text{cm} \times 61.1 \times 100}{\text{Total moisture of meat sample (mg)}}$$

equation 1

$$\text{WHC (\%)} = 100 - (\%)\ \text{free water}$$ equation 2

Cooking loss

Cooking losses were determined few hours after slaughter, according to the methodology proposed by Cason *et al.,* (1997). Raw breast meat samples were weighed, then steam-cooked in a water-bath at 85ºC for 30 minutes. After this procedure, the samples were cooled at room temperature and re-weighed.

$$\text{Cook loss (\%)} = \frac{(\text{W1} - \text{W2})}{\text{W1}} \times 100$$

where, W_1 = meat weight before cooking and W_2 = meat weight after cooking

Thiobarbituric acid reactive substance

Lipid oxidation was assessed in triplicate using the 2-thiobarbituric acid (TBA) method as described by Schmedes and Holmer (1989). Chicken breast meat samples (5g) were blended with 25 mL of 20% trichloroacetic acid solution (200 g/L of trichloroacetic acid in 135 mL/L phosphoric acid solution) in a homogenizer (IKA) for 30 sec. The homogenized sample was filtered with Whatman filter paper number 4, and 2 ml of the filtrate was added to 2 mL of 0.02 M aqueous TBA solution (3 g/L) in a test tube. The test tubes were incubated at 100°C for 30 min and cooled with tap water. The absorbance was measured at 532 nm using a UV-VIS spectrophotometer (UV-1200, Shimadzu, Japan). The amounts of thiobarbituric acid reactive substance were expressed as milligrams of malondialdehyde per kilogram of meat.

Microbiological assessment

Meat samples were collected from the breast muscle of each bird for microbial count and stored in a sterile container and refrigerated at 4ºC. The culture media were prepared 24 hours before collection of samples. For Lactobacilli count (Man Rogosa Sharpe agar was incubated anaerobically 48hr; Total bacteria count (nutrient agar, incubated aerobically 24h); *Escherichia coli* (Eosin methyl blue agar and Salmonella (*Salmonella shigella* Agar, incubated aerobically 24hr). The determination of the count's load was done according to the procedure of Feng *et al.,* (2002). One gram of the meat sample was added to a 9ml pre-reduced salt medium in other tubes. The suspension was prepared from 10^{-1} dilution and serial dilutions were done (10^{-2} - 10^{-5}), then serial dilution at 10^{-3} and 10^{-5} was used to culture the media. From the dilution, 0.1 ml of the sample was plated onto the appropriate medium for enumeration of bacteria. Discrete colonies on plates were counted using a colony counter and count estimated in logarithm number of bacteria per 1^{-}g sample (log10 CFU/g).

Statistical Analysis

Data were subjected to analysis of variance using SAS (2012) and the treatment means were separated using Duncan's Multiple Range Test of the same software at α0.05. Logarithmic (Log10) transformation was applied for microbial colony-forming unit (CFU) data.

RESULTS

Proximate compositions of experimental broiler diets (starter and finisher diets)

Proximate compositions (%) of starter and finisher diets are shown in Table 3. The values for dry matter, crude protein, ether extract, crude fiber and ash in the starter diet are 89.80, 24.15, 6.80, 5.60, and 5.10 respectively, with the corresponding values of 88.10, 21.87, 7.20%, 6.30, and 6.60 for finisher diet.

Table 3. Proximate composition of experimental diets (starter and finisher diets)

Parameter (%)	Starter diet	Finisher diet
Dry matter	89.80	88.10
Crude protein	24.15	21.87
Ether extract	6.80	7.20
Crude fiber	5.60	6.30
Ash	5.10	6.60
Nitrogen free extract	58.35	58.03

Growth performance of broiler chicken fed diets supplemented with synbiotic and/or organic acid at starter and finisher phases

The results on growth performance of broiler chicken fed diets supplemented with synbiotic and organic acid at starter and finisher phases are presented in Table 4. At the starter phase, diets did not influence the final weight (504.92-545.17g/bird) and weight gain (464.13-504.40g/bird) across the experimental groups. However, feed intake and feed conversion ratio were significantly ($P< 0.05$) influenced. Birds on the basal (1057.2g/bird), organic acid (1073g/bird), and synbiotic (1085.1g/bird) supplemented diets consumed more feed than those on the combination of organic acid + synbiotic diet (869.4g/bird). A lower feed conversion ratio (1.84) was recorded in birds fed blend of organic acid + synbiotic diet as compared with other treatment groups.

At the finisher phase, diets had no significant effect on the initial weight (504.92-545.17g/bird), final weight (1747.2-1863.6g/bird), and weight gain (1207.34-1318.43g/bird) of birds in the treatment groups. Birds on organic acid supplemented diet consumed lesser feed (1657.5g/bird) compared to those on the other diets. Feed conversion ratio (FCR) of birds fed synbiotic diet was similar to those placed on other dietary treatments. Improved FCR (1.38) was observed in birds that received organic acid supplemented diet, though, similar to the FCR (1.64) of birds fed synbiotic diet.

Table 4. Growth performance of broiler chicken fed organic acid and/or synbiotic supplemented diets at starter [0-21 days] and finisher [22-42 days] phases

Parameter	basal diet (control diet)	basal diet + organic acid	basal diet + synbiotic	basal diet + synbiotic + organic acid	SEM	P values
Starter phase						
Initial weight (g/bird)	40.80	41.28	40.77	41.08	0.65	0.932
Final weight (g/bird)	504.92	539.84	545.17	511.67	24.78	0.591
Weight gain (g/bird)	464.13	498.56	504.40	470.58	24.58	0.587
Feed Intake (g/bird)	1057.20^{a}	1073.00^{a}	1085.10^{a}	869.40^{b}	81.13	0.023
Feed conversion ratio	2.31^{a}	2.16^{a}	2.15^{a}	1.84^{b}	0.16	0.025
Finisher phase						
Initial weight (g/bird)	504.92	539.84	545.17	511.67	24.78	0.591
Final weight (g/bird)	1753.4	1747.2	1863.6	1747.2	73.26	0.618
Weight gain (g/bird)	1248.51	1207.34	1318.43	1235.5	59.59	0.607
Feed Intake (g/bird)	2659.10^{a}	1657.50^{b}	2234.00^{a}	2711.80^{a}	410.76	0.028
Feed conversion ratio	2.14^{a}	1.38^{b}	1.64^{ab}	2.20^{a}	0.33	0.026

^{ab} Means on the same row with different superscripts are significantly different (P<0.05)

Gut microbial load in broiler chicken fed organic acid and/or synbiotic supplemented diets

The results of gut microbial load in broiler chicken fed organic acid and synbiotic supplemented diets are presented in Table 5. Diets had a significant effect (P<0.05) on the *Escherichia coli*, total bacteria count, Salmonella *sp*, total viable count, and Lactic acid bacteria counts in broiler chicken on the experimental diets. The lowest *Escherichia coli* counts (0.57 x 10^5 CFU/mL) were recorded in birds fed blend of synbiotic plus organic acid supplemented diet as compared with the other groups. Total bacteria count in birds placed on the control diet (0.89 x 10^5 CFU/mL), synbiotic diet (0.92 x10^5 CFU/mL) and the blend (0.91x10^5 CFU/mL) were similar but significantly lower than those that received organic acid supplemented diet (1.03 x10^5 CFU/mL).

Salmonella counts (0.32 x10^5 CFU/mL) in birds fed with the control diet were similar to those on other diets. Total viable counts (TVC) of birds on the control (0.74x10^5 CFU/mL), synbiotic (0.74x10^5 CFU/mL) and the blend diets (0.74x10^5 CFU/mL) were similar but significantly (P<0.05) lower than the TVC (0.82x10^5 CFU/mL) in birds fed with organic acid supplemented diet. Increased lactic acid bacteria (LAB) counts (P<0.05) were observed in birds fed organic acid supplemented diet as compared with LAB counts in birds on the other dietary treatments.

Table 5. Gut microbial load of broiler chicken fed organic acid and synbiotic supplemented diets (×10^5 CFU/mL)

Parameter	basal diet (control diet)	basal diet + organic acid	basal diet + synbiotic acid	basal diet + synbiotic + organic acid	SEM	P values
Escherichia coli	0.76^a	0.71^a	0.69^a	0.57^b	0.010	0.025
Total bacteria count	0.89^b	1.03^a	0.92^b	0.91^b	0.002	0.0004
Salmonella *sp*	0.32^{ab}	0.28^b	0.37^a	0.36^a	0.003	0.039
Total viable count	0.74^b	0.82^a	0.74^b	0.74^b	0.002	0.008
Lactic acid bacteria	0.45^b	0.61^a	0.41^b	0.48^b	0.002	<.0001

[ab] Means on the same row with different superscripts are significantly different (P<0.05)

Gut morphology of broiler chicken fed organic acid and/or synbiotic supplemented diets

The results of gut morphology of broiler chicken fed organic acid and synbiotic supplemented diets are presented in Table 6. Diets had no influence on the villus height and villus width of birds on the dietary treatments. Lower epithelium height was observed in birds fed the control diet (22.64 µm) when compared with those on the other diets. Crypt depths of birds on the control diet (161.78 µm) were similar to those on the other diets. The epithelial muscular wall thickness of birds placed on the organic acid (163.16 µm) and synbiotic (166.32µm) supplemented diets were similar to those on the other diets. However, an epithelial muscular wall thickness of birds fed the blend (195.20µm) was significantly thicker (P < 0.05) than those on the control diet (121.98µm). The villus height: crypt depth ratio of birds fed with the control diet (4.48) was similar to those on other diets.

Table 6. Gut morphology of broiler chicken fed organic acid and synbiotic supplemented diets

Parameter	basal diet (control diet)	basal diet + organic acid	basal diet+ synbiotic	basal diet+ synbiotic + organic acid	SEM	P-values
Epithelium height (µm)	22.64^{b}	27.94^{a}	30.43^{a}	27.16^{a}	0.71	0.010
Villus height (µm)	719.97	682.75	651.64	647.31	19.84	0.555
Crypt depth (µm)	161.78^{ab}	123.05^{b}	137.57^{b}	192.97^{a}	7.32	0.019
Villus width (µm)	115. 67	129.58	124.37	123.52	3.75	0.634
Epithelial muscular wall thickness (µm)	121.98^{b}	163.16^{ab}	166.32^{ab}	195.20^{a}	8.91	0.070
Villus height: Crypt depth	4.48^{ab}	5.62^{a}	4.98^{a}	3.41^{b}	0.22	0.017

[ab] Means on the same row with different superscripts are significantly different (P<0.05)

Relative primal cuts, organ weight, and intestinal length of broiler chicken fed organic acid and/or synbiotic supplemented diets

The results of the relative primal cuts, organ weight, and intestinal length of broiler chicken fed organic acid, synbiotic and their combination are shown in Table 7. Diets had no significant effects (P>0.05) on primal cuts except the breast. Breast of birds placed on the synbiotic (25.42%) and the blend (25.13%) supplemented diets were similar to those on the other diets. However, breast of birds fed organic acid diet (26.22%) were significantly bigger (P<0.05) than those on the control diet (22.93%). The ranges for primal cuts values are: head (2.27%-2.41%), shank (4.02%-4.30%), wing (7.75%-8.10%), thigh (9.57%-10.58%), drumstick (9.47%-10.08%), back (13.00%-14.87%) and neck (4.31%-4.87%) respectively.

Diets had no influence on the liver (1.66%-2.01%), heart (0.49%-0.55%), gizzard (2.35%-2.61%), spleen (0.08%-0.11%), bursa of fabricius (0.10%-0.19%), duodenal length (33.00%-45.75%), and ileal length (81.50%-88.75%) of birds on the dietary treatments. The pancreas of bird fed control diet (0.34%) was significantly (P<0.05) higher than birds on organic acid (0.21%), synbiotic (0.24%), and the blend (0.22%) supplemented diets. Jejunal length in birds placed on the organic acid diet (86.00cm) and the blend diet (86.00cm) were similar but significantly longer than those that received synbiotic supplemented diet (82.50cm). However, birds on control diet had significantly (P<0.05) longer jejunal length (94.90cm) than those on the supplemented diets. Birds fed blend diet had increased caecal length (20.50cm) with the shorter length (19.33cm) recorded in birds on organic acid diet.

Table 7. Relative primal cuts (%), organ weights (%) and intestinal lengths (cm) of broiler chicken fed organic acid and synbiotic supplemented diets

Parameter	basal diet (control diet)	basal diet +organic acid	basal diet+ synbiotic	basal diet + organic acid + synbiotic	SEM	P-value
Live weight (g)	1911	1857.4	1927	1944.2	57.36	0.74
Head	2.27	2.34	2.34	2.41	0.13	0.89
Shank	4.02	4.24	4.23	4.30	0.21	0.80
Wings	8.10	7.83	7.75	7.76	0.2	0.58
Breast	22.93^{b}	26.22^{a}	25.42^{ab}	25.13^{ab}	0.9	0.1
Thigh	9.83	10.58	9.57	10.42	0.4	0.27
Drumstick	9.65	10.01	9.47	10.08	0.36	0.59
Back	13.47	13.00	14.87	13.74	1.12	0.68
Liver	2.00	1.83	1.66	2.01	0.12	0.17
Heart	0.55	0.49	0.49	0.53	0.03	0.57
Gizzard	2.40	2.35	2.61	2.35	0.12	0.4
Spleen	0.10	0.11	0.08	0.08	0.01	0.67
Pancreas	0.34^{a}	0.21^{b}	0.24^{b}	0.22^{b}	0.02	0.04
Bursa of fabricius	0.12	0.15	0.19	0.10	0.02	0.35
Duodenal length	34.13	33.00	33.25	45.75	3.44	0.52
Jejunal length	94.40^{a}	86.00^{b}	82.50^{c}	86.00^{b}	2.70	0.44
Ileal length	88.75	88.00	83.00	81.50	3.46	0.87
Caecal length	17.00^{d}	19.33^{c}	19.75^{b}	20.50^{a}	0.83	0.49

^{abcd} Means on the same row with different superscripts are significantly different (P<0.05), Results are expressed as percentage relatives to liveweight

Proximate composition and physicochemical properties of the meat of broiler chicken fed organic acid and/or synbiotic supplemented diets

The result of the single and combined effect of organic acid and synbiotic supplemented diets in broiler chicken is displayed in Table 8. The moisture content of birds fed the organic acid (72.17%), synbiotic (72.10%) and blend (72.39%) were similar but significantly higher than the basal diet (70.78%). The lowest ash content (1.25%) was recorded in birds fed the organic acid diet while highest value (1.73%) was found in the blend. Crude fat was the least (2.48%) in the blend and highest in basal diet. The crude protein of birds fed organic acid diet (22.96%) and synbiotic (22.87%) were similar but the blend (24.83%) was significantly (P<0.05) higher than the basal diet (21.92%). The cooking loss of synbiotic (22.73%) and the blend of organic acid plus synbiotic (23.73%) were similar but significantly (P<0.05) lower than the basal diet (32.75%) and organic acid (29.96%) diet. The lowest water holding capacity (64.67%) was recorded in birds fed the basal diet while the highest values (70.67%) were found in the synbiotic and blend supplemented diets. The TBARs of broiler chicken meat in birds fed organic acid (0.13 mgMDA/100g), synbiotic (0.12 mgMDA/100g) and the blend (0.14 mgMDA/100g) were similar but lower than the control diet (0.22 mgMDA/100g).

Table 8: Proximate composition and physicochemical properties of broiler chicken fed organic acid and synbiotic supplemented diets

Parameter (%)	basal diet (control diet)	basal diet + organic acid	basal diet + synbiotic	basal diet + organic acid + synbiotic	SEM	P-values
Moisture content	70.78^{b}	72.17^{a}	72.10^{a}	72.39^{a}	0.22	0.03
Ash	1.36^{c}	1.25^{d}	1.58^{b}	1.73^{a}	0.05	0.00
Crude Fat	4.50^{a}	3.19^{c}	3.73^{b}	2.48^{d}	0.17	0.00
Crude Protein	21.92^{c}	22.96^{b}	22.87^{b}	24.83^{a}	0.28	0.00
Cooking loss	32.75^{a}	29.96^{b}	22.73^{c}	23.73^{c}	0.49	0.02
Water holding capacity	64.67^{c}	68.33^{b}	70.33^{a}	70.67^{a}	0.10	0.01
TBARS (mgMDA/100g)	0.22^{a}	0.13^{b}	0.12^{b}	0.14^{b}	0.02	0.00

^{abcd} Means on the same row with different superscripts are significantly different (P<0.05)

TBARS- Thiobarbituric acid reactive substance

Microbial load in the meat of broiler chicken fed organic acid and/or synbiotic supplemented diets

The results of microbial load in the meat of broiler chicken fed organic acid and synbiotic supplemented diets are presented in Table 9. Diets had no significant influence (P>0.05) on the total bacteria count and total viable count. The total Lactobacillus counts (0.58×10^4 CFU/g) in birds fed synbiotic diet and the combination of synbiotic plus organic acid (0.65×10^4CFU/g) were similar but significantly (P<0.05) higher than the control diet (0.34×10^4 CFU/g) and organic acid diet (0.20×10^4 CFU/g). Total Salmonella count was lowest (0.32×10^3 CFU/g) in the blend and highest (0.86×10^3 CFU/g) in organic acid diet. The Total *Escherichia coli* count (2.80×10^3 CFU/g) in the control diet was similar to the other diets.

Table 9. Microbial load (CFU/g) of broiler chicken meat fed organic acid and/or synbiotic supplemented diets

Parameter	basal diet (control diet)	basal diet + organic acid	basal diet + synbiotic	basal diet + organic acid + synbiotic	SEM	P values
TLC ($\times 10^4$)	0.34^b	0.20^c	0.58^a	0.65^a	0.04	0.00
TSC ($\times 10^3$)	0.57^c	0.86^a	0.68^b	0.32^d	0.05	0.00
TECC ($\times 10^3$)	2.80^{ab}	2.21^b	3.21^a	2.28^b	0.13	0.01
TBC ($\times 10^6$)	3.35	3.37	3.32	3.04	0.10	0.63
TVC ($\times 10^6$)	2.35	2.38	2.41	2.33	0.05	0.97

^{abc} Means on the same row with different superscripts are significantly different (P<0.05)

TLC- Total Lactobacillus count TSC- Total Salmonella count TECC- Total *Escherichia coli* count TBC- Total Bacteria count

TVC- Total Viable count

DISCUSSION

The beneficial effects of feed additives in poultry nutrition cannot be overemphasized. The present study shows that the supplementation of broiler chicken diet with the blend of organic acid plus synbiotic resulted in lowered feed conversion ratio at the starter phase. This result agrees with the findings of Salah *et al.,* (2018) who averred that supplementing diets with synbiotic (1 g/kg) or synbiotic plus organic acid (2 g/kg) could improve the growth performance of broiler chickens. Elsagheer *et al.,* (2020) reported that 0 to 42 days' quails had a better feed conversion ratio when synbiotic was introduced at 1g/kg inclusion rate. In the present study, the improvement in the feed conversion ratio of birds that received diets supplemented with the blend could be attributed to the complementary and synergistic effects of prebiotic and probiotic in the diets. Similarly, the favourable environment created by reduced pH probably enhanced the proliferation of beneficial microflora in the alimentary tract of the birds.

Although there was no significant increase in the weight gain of the broiler chicken fed the additive diets but additions of organic acid or synbiotic singly or their combination in broiler diet had no adverse effects on broiler performance.

At the finisher phase, lowest feed intake and improved feed conversion ratio were observed in birds fed organic acid supplemented diet. This result agrees with the findings of Salah *et al.,* (2018), who reported decreased feed intake and bodyweight gain with dietary supplementation of organic acid (1 g/kg) when compared with other treatment groups. But the authors recorded a considerable improvement in the overall feed conversion ratio with the synbiotic plus organic acid diet when compared with the control and organic acid-supplemented groups. However, Agboola *et al.,* (2019) reported that growth performance of 21-day broiler chicken fed varying levels (0.1-0.5%) of butyric acid supplemented diets was not statistically different from the control group. According to Adil *et al.,* (2010), chicks fed diet supplemented with organic acids showed a significant improvement in the feed conversion ratio with decreased feed consumption as against the chicks fed the control diet. The reduction in the feed intake might be due to the strong taste associated with the organic acids which would have decreased the palatability of the feed, thereby reducing feed intake. Attia *et al.,* (2013) reported that feed intake from 15 to 42 days of age of Japanese quail decreased with significantly improvement in feed conversion ratio compared to the control group when acetic acid was introduced at 1.5, 3, and 6% inclusion rates. Several authors found that growth rate and feed conversion ratio of broilers and ducks were improved due to supplementation of organic acid and/or salts such as formic acid (Vogt *et al*., 1981), formate and propionate (Paul *et al*., 2007), and acetic acid (Attia *et al*., 2013) respectively.

The present study reveals that supplementing broiler chicken diet with the synbiotic plus organic acid resulted in decreased *Escherichia coli* counts. This contradicts the findings of Omidiwura *et al.,* (2018), who reported no significant differences in the microbial load of turkey poults fed prebiotic, probiotic, or synbiotic supplemented diets when compared with the control groups. Fouladi *et al.,* (2014) showed that the diets containing organic acid presented significant effects on the *Escherichia coli* population in ileum compared with the control group in female Japanese quail. This disagrees with the findings of Agboola *et al.,* (2016), who revealed that there were no differences in the microbial populations up to 600mg\kg inclusion level of probiotic in broiler diets. Thus, from the present result, it was observed that synbiotic and organic acid fed singly did not have remarkable effect on *Escherichia coli* count. However, the additive effects of synbiotic plus organic acid resulted in reduced *Escherichia coli* count as observed in this study. Organic acid alters the gut microflora either directly by killing through cell-wall penetration or indirectly by modifying pH and reducing the numbers of pathogenic bacteria, increasing acid tolerant beneficial species such as

Lactobacilllus *sp.* and reducing competition for nutrients by the altered microbes (Czerwiński *et al*., 2010; Boroojeni *et al*., 2014).

In this study, the results reveal highest Lactic acid bacteria and reduced Salmonella *sp* in organic acid groups. This is contrary to Omidiwura *et al.,* (2018), who revealed that diets had no significant effect on total bacteria count, Salmonella, *Escherichia coli,* and lactic acid bacteria at the ileal section of turkey poults when prebiotic, synbiotic, and probiotic were fed to turkey poults. However, the result is similar to the findings of Thirumeignanam *et al.,* (2006), who reported an increase in Lactobacilli load because of dietary acidification. Gunal *et al.,* (2006) reported that the addition of probiotics alone or a combination of probiotics with organic acid mixture decreased ileal and caecal gram-negative bacteria counts at 42 days. A possible explanation is the increase of Lactobacilli counts in organic acid supplemented diet as reported by Biggs and Parsons (2008), is through modification of the gut microbiome by destroying the cell wall and/or modifying the pH by lowering the pH thereby making the conditions unfavourable for the pathogenic bacteria.

Gut morphological indices like the villus height, villus length, crypt depth, villus width, epithelial thickness, etc. are considered as criteria to reflect the small intestine morphology and absorptive capacity (Montagne *et al*., 2003). The epithelium tissue serves as a protective barrier for body as an interface with the environment. This structural and functional integrity of this epithelium is vital for normal gut health. Lower epithelium height was observed in birds fed the control diet when compared with those on the other diets but the epithelial muscular wall thickness of birds fed the blend was thicker than the control group. This result is contrary to the findings of Omidiwura *et al.,* (2018), who averred that the epithelial cell thickness of the ileal section of turkey poults was not significantly influenced by prebiotic, probiotic, or synbiotic supplementation. The villi are associated with total lumina absorptive area and subsequent satisfactory digestive enzymes action and higher transport of nutrients. In the current study, diet had no significant influence on the villus height and villus width. This result corroborates with the findings of Agboola *et al.,* (2015), who reported that diet had no effects on villus width when organic acid and probiotic supplemented diets were fed to broiler chickens. On contrary, Salmanzadeh (2013), reported that the dietary butyric acid addition in Japanese quails also induced highly significant increases in the jejunal villus dimensions (height and width) since the end of the staring period (day 21) persisting over the growing period (day 42).

Villus crypt is regarded as the villus factory and deeper crypt indicates fast tissue turnover to permit renewal of the villus as needed in response to normal sloughing or inflammation from pathogens or their toxins and high demand for tissue (Yason *et al*., 1987). Agboola *et al.,* (2019) reported no significant differences in the crypt depths of broiler chicks fed graded levels of butyrate supplemented diets. Contrary to the present result, Salah *et al.,* (2018), recorded a significant increase in the jejunal villus height when synbiotic and combination of synbiotic and organic acid diets were fed to broiler chickens. Also, Awad *et al.,* (2009) reported reduced ileal crypt depth of broilers fed synbiotic supplemented diet when compared with the control groups. However, Beski and Al-Sardary (2015) asserted that dietary supplementation of probiotics and synbiotic had no significant effect on crypt depth as compared with the control group in broilers.

The villus height to crypt depth ratio is a useful criterion for estimating the digestive capacity of the small intestine. A high villus height to crypt depth ratio indicates a decreased turnover of the intestinal mucosa. A slower turnover rate of the intestinal epithelium results in a lower maintenance requirement, which can finally lead to a higher growth rate or growth efficiency of the animal (Van Nevel *et al*., 2005). In this study, higher villus height to crypt depth ratio was observed in synbiotic or organic acid groups, though similar to birds fed the control diet. El-Wahab *et al.,* (2020). reported higher villus height to crypt depth ratio in Japanese quails fed 3.5% supplemented yeast diet compared to those fed non supplemented diets. Wu *et al.,* (2004) affirmed that an increase in the villus height to crypt depth ratio is associated with better nutrient absorption, decreased secretion in gastrointestinal tract, improved disease resistance, and faster growth.

The outcome of this study reveals that the single and combined effect of organic acid and synbiotic affected breast meat of broiler chicken. This is in agreement with the findings of Aksu *et al.,* (2007) who reported significant improvement in the breast weight of the bird with organic acid supplementation. Such a positive impact of organic acid on breast muscle might be due to a reduction of pH values in the feed and digestive tract. This resulted in reduced pathogenic organisms which are sensitive to low pH or selectively increasing the acid loving Lactobacillus *sp.* and a direct antimicrobial effect which help in nutrient utilization (Ghazala *et al.,* 2011). Abdel-Raheem and Abd-Allah (2011) also reported a significant increase in the carcass weight and dressing percentage of broilers fed synbiotic supplemented diets when compared with other diets. The non-significance in the carcass yields from the broiler chicken irrespective of the additives could be due to the fact that the nutritional components of the feed offered to the birds were similar, thus facilitating similar carcass and primal cut development under different dietary treatments. In contrast, Pelicano *et al.,* (2003) reported that chickens fed probiotic supplemented diet had higher yields of carcass and shanks.

Diet had no significant effect on the relative organ weight among the treatments except in the pancreas, jejunal length, and cecal length. Lower pancreas weight was recorded in supplemented diets as compared with the control group. Contrary to this finding, Fateme *et al.,* (2016) reported improved relative weight of pancreas with the inclusion of organic acid in broiler diet. A relative increase in pancreas could be an indication that birds possess a higher immune status to fight against pathogens causing infectious diseases. The present study shows no improvement in gizzard weight. Ashayerizadeh *et al.,* (2009) also reported that weight of gizzard was not affected by the inclusion of synbiotics in broiler diet. In the present study, caecal length was the longest when broiler was fed with the blend. This could be as a result of the effect of synbiotic and organic acid on caecal microflora proliferation which influences gene expression of caecal tonsils (Slawinska *et al*., 2016).

The proximate composition of the broiler chicken meat from the supplemented diets was improved compared with the control group. This is following the findings of Thakur *et al.,* (2017) who reported that feeding broilers with synbiotics had a significant effect on the crude protein, ash contents, and ether extract of the breast meat. The improvement in crude protein content of the breast meat of birds that received the blend could be due to the intestinal uptake of glucose and amino acid interrelationships which have been considered pivotal to broiler performance (Throat *et al.,* 2015). The present study also shows that feeding organic acid or synbiotic supplemented diet to broiler chicken reduced the crude fat and increased the moisture and crude protein contents. This result agrees with the findings of Kadim *et al.,* (2008) who reported an increase in crude protein and moisture when broilers were fed organic acid supplemented diet. The low-fat contents of the birds fed combination of the feed additives imply that the birds would have less accumulation of fat in their tissues which would in turn reduce the rate of lipid oxidation as reflected in their level of thiobarbituric acid reactive substances (TBARS). In addition, the consumers are provided with healthy meat as people tends to move away from animal fat consumption (Ritzoulis *et al.,* 2010). Also, this reduced fat in birds fed combination of synbiotic plus organic acid had a positive effect on the cooking loss of the meat. A high fat meat melts and shrinks during cooking (as most of the fat is lost to cook out (brooth) which is of poor economic benefits to the producer.

The result of the physicochemical properties implies that the combination of organic acid and synbiotic diet had positive effect on the quality of the birds through increased water holding capacity and decreased cooking loss. The decreased cooking loss of breast fillets of all the supplemented diets observed in this study might be associated with the reduced fat content of the meat; while the high water holding capacity could be a result of the synergy effect of the synbiotic additive as reported by Zhou *et al.,* (2010) who recorded a high water holding capacity with synbiotic supplemented diet. Cooking loss is not only related to sensory properties but also tied to the loss of soluble nutrients and market value of meat (Leipeng *et al*., 2016). In this study, the inclusion of synbiotic singly and in combination with organic acid in broiler chicken diet lowered cooking loss of meat. Menconi *et al.,* (2014) and Cheng *et al.,* (2017) reported a decrease in cooking loss with organic acid or synbiotic supplementation. Water holding capacity is the ability of the meat to hold its inherent moisture during external force such as slicing, mincing and pressing (Pearce *et al*., 2011). This study reveals that organic acid and synbiotic combination improved water holding capacity of the broiler meat. Menconi *et al.,* (2014) also reported that organic acid supplementation had significant effect on the water holding capacity of meat. The inclusion of synbiotic and organic acid in broiler diet helps to partially solubilize or dissociate protein in muscle and this changes the pH which leads to the expansion of cellular structures that results in water absorbability of the meat (Hultin *et al*., 2007). The TBARS is determined in fresh meat to estimate the degree of rancidity (lipid oxidation) in meat. Lipid oxidation is one of the most important causes of deterioration of poultry meat quality and can lead to decreased shelf life. (Lorenzo and Gomez, 2012). The level of malondialdehyde (MDA) is used as an indicator of the extent of lipid oxidation in meat. Oxidation in meat could increase juice loss by reducing hydrolysis sensitivity and water reservation (water holding capacity) among myofibrils (Jun Li *et al.,* 2019). The TBARS index of the breast fillet fed additives presents somewhat a similar sequence, with similar oxidation levels equivalent to almost above half of the TBARS value of the control sample. This is an indication that the birds fed the supplemented diets had reduced MDA accumulations in their breast muscle compared with the birds fed with the basal diet. The trend observed in this study was also reported by Luiz Gustavo *et al.,* (2012) who reported a decreased in TBAR index when broilers were fed with different feed additives. Also, Cheng *et al.,* (2017) and Untea and Panaite (2020) reported a decreased in the MDA accumulation in the thigh and breast muscles of broilers fed synbiotic supplementation diet.

The microbial safety and quality of poultry meat are important to the producers, retailers, and most importantly the consumers (Mead, 2004). Total viable count (TVC) is a general measure of the microbiological status of meat and in raw poultry, it indicates hygienic conditions of processing plants under which the meat is processed (Meat Industry Guide, 2017). A high load of TVC increases the risk of microbial spoilage but TVC results and the number of pathogens present may not always be related (Javadi and Safarmashaei, 2011). The microbial counts (TVC) presented in this study was within the maximum limit of $\leq 10^5$-10^7CFU/g recommended/regulated by many countries (Hye-Jin *et al*., 2018). However, what is obtained in this study is lower than $3.7x10^6$ CFU/g recorded for breast muscle of chicken (Hassanien *et al.,* (2016) and higher than 2.1×10^3 obtained by Daoud *et al.,* (2012). Salmonella is an important pathogen of meat contamination (Wabeck, 2002). In this study, reduced total Salmonella count was recorded in the blend group. Van Immerseel *et al.,* (2006) also reported that organic acid inclusion in broiler diet diminished the population of Salmonella in broiler. This is attributed to the antimicrobial properties of organic acid which decrease the intracellular pH thereby reducing the invasion of Salmonella in the meat. Total *Escherichia coli* count was at its lowest when broiler was fed organic acid and its combination with synbiotic though similar with the control group. Ateya *et al.,*

(2019) reported that combination of synbiotics and organic acid weakened the inflammatory response in chicken challenged with *E. coli.* The presence of pathogenic microorganisms such as *Escherichia coli, Salmonella spp,* etc. in this study could be attributed to the fact that meat is enriched with all nutrients required for the growth of bacteria in adequate quantity (Jahan *et al*., 2015).

CONCLUSIONS

The outcome of the present study shows that broiler chicken fed dietary supplementation of organic acid, synbiotic and the blend had improved feed conversion ratio which resulted in improved relative primal cuts, relative organ weights, and intestinal length of broiler chicken. Supplementation of organic acid plus synbiotic lowered *E. coli* and Salmonella counts with highest counts of lactic acid bacteria in broiler chicken. Improved gut integrity and meat quality of broiler chicken were observed in birds fed with organic acid diet and the combination of organic acid plus synbiotic diet. Therefore, these feed additives, at the inclusion rate of 1g/kg organic acid singly or plus 0.5g/kg synbiotic, can conveniently replace antibiotics used as growth promoters in broiler chicken production.

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