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EFFECT OF HUMIC ACID SUPPLEMENTATION ON GROWTH RESPONSE, GUT MORPHOLOGY AND MICROBIAL LOAD IN BROILER CHICKENS

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ABSTRACT

One-day old unsexed Arbor Acre plus broiler chicks (n = 200) were randomly allotted to 5 treatments (diets) consisting of 5 replicates with 8 birds each: Diet 1: basal diet; Diet 2: basal + antibiotic at 0.2g.kg^{-1} diet (21 days); Diet 3: basal + humic acid at 1 g.kg^{-1} diet (21 days); Diet 4: basal + antibiotic at 0.2 g.kg^{-1} diet (42 days); and Diet 5: basal + humic acid at 1 g.kg^{-1} diet (42 days). Performance parameters were measured.
After 42 days, the birds on antibiotic supplemented diet had significantly ($p < 0.05$) higher weight gain (2251.75 g per bird) than those on the control diet (2112.32 g per bird). Similar final weights were recorded for birds on antibiotic and humic acid diets fed for 21 days and humic acid diet fed for 42 days respectively. Similar trend was followed in the weight gain of the birds on dietary treatments. The cost of feed per kg weight gain of birds fed with humic acid supplemented diets for 21 days (Nigerian naira, ₦ 94.18) was similar to those on other treatment groups. Improved ($p < 0.05$) villus height (724.99 µm), villus width (68.31 µm), crypt width (29.20 µm) and villus height to crypt depth ratio (0.79 µm) were recorded in birds placed on humic acid supplemented diet though similar to those on the control diet. Highest total bacteria count was recorded in birds on the control diet, while the least was in those fed antibiotic diet. Total *E. coli* and coliform counts were higher in birds on the control diets compared with those on the antibiotic and humic acid supplemented diets. In conclusion, dietary humic acid (1 g.kg^{-1}) inclusion into the diet of broiler chickens is a viable alternative to antibiotic growth promoters for improved performance and gut integrity in broiler chickens.

Key words: broiler chicks; humic acid; gut morphology; microbiome; growth performance

INTRODUCTION

In animal husbandry, antimicrobial feed additives are used worldwide to boost the economy and ecology of animal production by increasing the growth rate, decreasing feed expenditure per benefit, and decreasing disease risk (Islam *et al.*, 2005). The push towards improving food safety has, therefore, necessitated the feeding approaches to reduce the risk and economic effect of digestive diseases. Digestive disorders pose one of the key challenges in poultry farms, especially in the first phase of rearing, often leading to unregulated use of antibiotics to avoid huge animal losses. Nonetheless, the indiscriminate use of antibiotics in poultry feeds has become unacceptable due to residues in animal products, such as meat and eggs, and the emergence of antibiotic-resistant bacterial species in humans. As a result of this, antibiotics have been banned in the European Union as a growth-promoting agent for poultry (Mutus *et al.*, 2006) and other countries (Guban *et al.*, 2006; NAFDAC, 2018). Many additives have been tested as growth promoters to prevent unnecessary use of antibiotics or at least minimize or substitute their use in feed while maintaining successful animal production in order to produce

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healthy edible products (Islam *et al.*, 2005; Gomez *et al.*, 2012). Numerous of the alternatives used include probiotics, prebiotics, herbal compounds and organic acids. Most of these substances exert their influence by affecting processes in digestion and the gastrointestinal flora. Amongst these alternatives, it has been stated that the effect of humic acid (an organic acid) has a positive impact on growth efficiency of birds (Seyed *et al.*, 2012).

Humic acids (HA) are naturally occurring decomposed organic constituents of soil and lignite that are complex mixtures of polyaromatic and heterocyclic chemicals with multiple carboxylic acid side chains (MacCarthy, 2001). Humic acid usage in poultry to replace antibiotics has gained widespread interest (Mutus *et al.*, 2006). It has been reported to have immune system improvement (Hooge, 2004), anti-inflammatory activity (Yang *et al.*, 1996), antiviral properties (Huck *et al.*, 1991) and antithyroid effects (Islam *et al.*, 2005). Humic acid used in poultry feed and water has been found to promote growth (Kocabagli *et al.*, 2002). It also has a beneficial impact on the functioning the liver and consequently decreases mortality and increases poultry performance (Islam *et al.*, 2005). HA has shown a strong affinity to bind specific substances, such as heavy metals (Madronova *et al.*, 2001) and aflatoxins (Van Rensburg *et al.*, 2006). Such physiochemical properties of HA can also be responsible for some of the tissue effects, including heavy metal removal (Madronova *et al.*, 2001), desmutagenic effects (mutagens extracellular interception) (Sato *et al.*, 1987) and antibacterial effects (Riede *et al.*, 1991). Against this background, the objective of this study was to evaluate the effect of humic acid supplemented diets on growth performance, gut morphology and intestinal microbiota of broiler chickens.

MATERIAL AND METHODS

Experimental Site

The experiment was conducted in the poultry unit of the University of Ibadan teaching and research farm Oyo State, located within latitude 3 and 5°N; between longitude 7°E and 9.3°E, with an average temperature of between 24 and 25 °C and rainfall figures varying from an average of 800 mm at the onset of heavy rains to 1500 mm at its peak. This study complied with the University of Ibadan ethics requirements for animal care and handling.

Experimental material

Humic acid was provided by Rising Yuera International Inc. 11/F Suit 1111 Burgundy Empire Tower, corner Garnet and Sapphire Sts., Ortigas center, Pasig City, Philippines and its certified composition is as follows: (1) sodium humate salt – 14.0 %; (2) humic acid (DM basis) – 76.0 %; (3) organic matter – 92.0 %; (4) water solubility – 98.0 %; (5) pH (in solution) – 10; (6) particle size – 80 mesh; (7) colour – dark brown (8); physical consistency – powder.

Experimental diets and management of birds

Two hundred (200) one-day old unsexed Arbor acre plus broiler chickens were purchased from a reputable commercial hatchery within Oyo state, Nigeria. The birds were weighed, tagged, and randomly allocated into five dietary treatments comprising of five replicates of eight birds per replicate in a deep litter system. The chicks were given vitamins (anti-stress) from the first to the third day of age of arrival, as well as before, during and after vaccination. The birds were vaccinated against Newcastle disease and infectious bronchitis as appropriate. Experimental diets, in mash form, and fresh water were provided *ad-libitum* during the study period that lasted for 6 weeks. Lighting was provided without restriction throughout the study period.

Experimental layout

The basal diet was a corn-soybean diet formulated to meet the nutrient requirements (NRC 1994) for broiler starter (0 – 3 weeks) and broiler finisher (4 – 6 weeks) (Table 1). The feed supplements/additives (humic acid and antibiotics oxytetracycline) were added over the top to the diet as follows:

1. Basal diet (negative control),
2. Basal diet + antibiotics oxytetracycline (positive control) at inclusion level of 200 mg.kg^{-1} diet, fed for 21 days,
3. Basal diet + humic acid at inclusion level of 1000 mg.kg^{-1} diet, fed for 21 days,
4. Basal diet + antibiotics oxytetracycline (positive control) at inclusion level of 200 mg.kg^{-1} diet, fed for 42 days.
5. Basal diet + humic acid at inclusion level of 1000 mg.kg^{-1} diet, fed for 42 days.

The birds were distributed to the dietary treatments in a completely randomized design.

Parameters measured and data collection
Performance parameters

The weight gain was calculated by subtracting the initial weight from the final weight taken weekly at the end of days 21 and 42 of the experiment, respectively. To obtain the feed conversion ratio, the feed supplied was weighed and served, left over feed was collected and weighed, the difference of which gave the value of feed intake. The feed conversion ratio (FCR) was then computed by dividing daily feed intake by daily weight gain.

Gut morphology measurements

At days 21 and 42 of the experiment, 2 birds per replicate (10 birds per treatment) were randomly selected, weighed and slaughtered. Samples of ileum (3 cm segments) between Meckel's diverticulum and ileo-caeco-colonic junction were severed and immersed into a phosphate-buffered formalin solution. Transverse sections were cut (3 µm), stained by haematoxylin-eosin and analysed under a light microscope to determine morphometric indices. The morphometric variables measured are villus height, crypt depth and villus width at the top and the base, crypt height, epithelial height and goblet cell count. The villus height to crypt depth ratio was also calculated.

Microbial analysis

The standard microbiological plate technique of Harrigan and MacCance (1966) was used for the analysis of the samples. The intestinal contents were analysed for Total Heterotrophic Count, Total *E. coli* and Total coliforms. The setup was incubated at 35 ± 2 °C for 24 – 48 hours, after which observations

Table 1. Gross composition (%) of experimental diets for broiler chickens

Feed Ingredient	Starter (0 – 21 days)			Finisher (22 – 42 days)		
	T1	T2	T3	T1	T2	T3
Maize	48.00	48.00	48.00	50.50	50.50	50.50
Soybean meal	38.00	38.00	38.00	37.00	37.00	37.00
Fishmeal	5.00	5.00	5.00	2.00	2.00	2.00
Soy Oil	5.00	5.00	5.00	6.50	6.50	6.50
Dicalcium Phosphate	2.00	2.00	2.00	2.00	2.00	2.00
Lime Stone	1.00	1.00	1.00	1.00	1.00	1.00
Salt	0.25	0.25	0.25	0.30	0.30	0.30
Methionine	0.25	0.25	0.25	0.30	0.30	0.30
Lysine	1.00	1.00	1.00	0.20	0.20	0.20
Vit./Min. Premix	0.25	0.25	0.25	0.25	0.25	0.25
Oxytetracycline	-	+	-	-	+	-
Humic Acid	-	-	++	-	-	++
Total	100.00	100.00	100.00	100.00	100.00	100.00
Nutrient Composition						
Crude Protein (%)	24.1	24.1	24.1	20.7	20.7	20.7
Metabolizable Energy ($MJ.kg^{-1}$)	12.65	12.65	12.65	13.05	13.05	13.05
Fat (%)	3.55	3.55	3.55	3.34	3.34	3.34
Crude Fibre (%)	3.96	3.96	3.96	3.94	3.94	3.94
Calcium (%)	1.17	1.17	1.17	0.99	0.99	0.99
Total Phosphorus (%)	0.87	0.87	0.87	0.78	0.78	0.78
Non-Phytate Phosphorus (NPP) (%)	0.48	0.48	0.48	0.48	0.48	0.48
Ca/NPP	0.21	0.21	0.21	0.19	0.19	0.19

Starter: T1 – Control (basal diet); T2 – basal diet + antibiotic fed for 21 days; T3 – basal diet + humic acid fed for 21 days.
Finisher: T1 – basal diet; T2 – basal diet + humic acid fed for 42 days; T3 – basal diet + humic acid fed for 42 days.
(-) = Absent; (+) = Antibiotic at 0.02 %; (++) = humic acid at 0.10 %

were made and recorded. The media used were: nutrient agar, eosin methylene blue and MacConkey agar (Oxoid, UK). They were prepared according to the manufacturers' instruction on each medium jar and then sterilized by autoclaving at 121 °C for 15 minutes and cooled down before use.

Proximate analysis

The proximate composition of the experimental diets was done using the methods described by AOAC (2000). Experimental diets were analysed for nitrogen (N) by the Kjeldahl method and crude protein (N x 6.25) (AOAC, 2000; Method number: 982.30).

Statistical analysis

Data obtained were analysed using descriptive statistics and ANOVA of SAS (SAS, 2012). Significant level of $P = 0.05$ was used. The treatment means were compared using Duncan's Multiple Range Test (Duncan, 1955).

RESULTS

Effect of humic acid supplementation on performance of broiler chickens (0 – 21 days)

The results of the humic acid supplementation on performance of broiler chickens at the starter phase are shown in Table 2. At 21 days of feeding, there were no significant differences observed in the final weight and weight gain of the birds. However, significant differences ($p < 0.05$) were observed in the Feed Intake (FI) and Feed Conversion Ratio (FCR) of the birds at the end of the 21 days feeding trial. The highest FI (1160.0 g per bird) was recorded in birds on the control diet, and this was significantly different from those on antibiotic (1100.0 g per bird) and humic acid (1099.29 g per bird) supplemented diets respectively, which had similar FI. Birds on humic acid supplemented diet had FCR (1.66) similar to those on the control (1.68) and antibiotic (1.58) diets.

Effect of humic acid supplementation on performance characteristics of broiler chickens (0 – 42 days)

The results of humic acid supplemented diets on performance of broiler birds after 42 days of feeding are presented in Table 3. After 42 days of continuous feeding of experimental diet, there were no significant differences observed in the parameters measured except weight gain. The birds on antibiotic supplemented diet had significantly ($p < 0.05$) higher gain (2251.75 g per bird) than those on the control diet (2112.32 g per bird). However, birds placed on humic acid supplemented diet (2195.96 g per bird), had similar weight gain with those in control and weight antibiotic groups.

Comparative effect of humic acid supplementation on performance and cost analysis of broiler chickens (0 – 42 days)

The results of the performance characteristics of broiler chickens placed on humic acid supplemented diets for 0 – 21 and 0 – 42 days are presented in Table 4. There was no significant difference observed in the FCR of birds placed on the experimental diets. The birds fed antibiotic supplemented diet for 42 days recorded a final weight of 2295.83 g per bird, which was statistically ($p < 0.05$) higher than those on the control diet (2157.90 g per bird). Similar final weights were recorded for birds on antibiotic and

Table 2. Performance indices of broiler chickens fed humic acid supplemented diets (0 – 21 days)

Parameters	T1	T2	T3	SEM	*P*-value
Initial weight (g per bird)	45.58 ± 1.08	44.18 ± 0.93	44.95 ± 0.75	0.42	0.099
Final weight (g per bird)	739.00 ± 42.18	742.38 ± 52.43	707.75 ± 51.21	21.83	0.487
Weight gain (g per bird)	693.43 ± 42.02	698.20 ± 52.24	662.80 ± 51.93	21.90	0.485
Feed intake (g per bird)	1160.0 ± 22.36^{a}	1100.0 ± 53.03^{b}	1099.29 ± 51.78^{b}	19.99	0.043
FCR	1.68 ± 0.09^{a}	1.58 ± 0.07^{b}	1.66 ± 0.10^{ab}	0.04	0.021

Means within the same row with the same superscript are not significantly different ($p > 0.05$). T1 – Control (basal diet); T2 – basal diet + antibiotic fed for 21 days; T3 – basal diet + humic acid fed for 21 days. FCR = Feed Conversion ratio. SEM – Standard Error of Means.

Table 3. Performance indices of broiler chickens fed humic acid supplemented diets (0 – 42 days)

Parameters	T1	T2	T3	SEM	*P*-value
Initial weight (g.b^{-1})	45.57 ± 1.08	44.08 ± 0.64	43.85 ± 1.07	0.43	0.289
Final weight (g.b^{-1})	2157.90 ± 215.25	2295.83 ± 127.02	2239.81 ± 124.96	72.15	0.423
Weight gain (g.b^{-1})	2112.32 ± 215.21^{b}	2251.75 ± 126.48^{a}	2195.96 ± 125.02^{ab}	72.08	0.041
Feed intake (g.b^{-1})	4754.29 ± 25.55	5021.90 ± 706.90	4821.90 ± 304.53	198.85	0.625
FCR	2.27 ± 0.21	2.24 ± 0.39	2.20 ± 0.12	0.12	0.916

Means within the same row with the same superscript are not significantly different ($p > 0.05$). T1 – Control (basal diet); T2 – basal diet + antibiotic fed for 42 days; T3 – basal diet + humic acid fed for 42 days. FCR = Feed Conversion ratio. SEM – Standard Error of Means.

Table 4. Comparative effect of humic acid supplemented diets on performance and cost analysis of broiler chickens (0 – 42 days)

Parameters	T1	T2	T3	T4	T5	*P*-value
Initial weight (g.b^{-1})	45.58 ± 1.08	44.18 ± 0.93	44.95 ± 0.75	44.08 ± 0.64	43.85 ± 1.07	0.390
Final weight (g.b^{-1})	2157.90 ± 215.25^{b}	2230.40 ± 158.30^{ab}	2269.04 ± 71.97^{ab}	2295.83 ± 127.02^{a}	2239.81 ± 124.96^{ab}	0.033
Weight gain (g.b^{-1})	2112.32 ± 215.21^{b}	2186.22 ± 158.84^{ab}	2224.08 ± 72.30^{ab}	2251.75 ± 126.48^{a}	2195.96 ± 125.01^{ab}	0.032
Feed intake (g.b^{-1})	4754.29 ± 25.55^{ab}	4651.43 ± 73.26^{b}	4758.10 ± 268.22^{ab}	5021.90 ± 906.90^{a}	4821.90 ± 304.53^{ab}	0.03
FCR	2.27 ± 0.21	2.13 ± 0.14	2.14 ± 0.13	2.24 ± 0.39	2.20 ± 0.12	0.84
Feed cost per kg (₦)	191.78 ± 0.00^{e}	193.28 ± 0.00^{d}	209.28 ± 0.00^{b}	194.10 ± 0.00^{c}	218.83 ± 0.00^{a}	<0.0001
Feed cost per kg per bird (₦)	27.40 ± 0.00^{e}	27.61 ± 0.00^{d}	29.89 ± 0.00^{b}	27.72 ± 0.00^{c}	31.26 ± 0.00^{a}	<0.0001
Feed Cost per kg weight gain (₦)	91.48 ± 8.40^{b}	88.77 ± 6.20^{b}	94.18 ± 3.01^{ab}	86.43 ± 5.14^{b}	99.91 ± 5.78^{a}	0.018

Means within the same row with the same superscript are not significantly different ($p > 0.05$). T1 – Control (basal diet); T2 – basal diet + antibiotic fed for 21 days; T3 – basal diet + humic acid fed for 21 days; T4 – basal diet + antibiotic fed for 42 days; T5 – basal diet + humic acid fed for 42 days. FCR = Feed Conversion ratio. SEM – Standard Error of Means.
Note: *₦ = Nigerian naira*; *$1 = ₦ 380 (at the period of experiment).*

Table 5. Gut morphological indices of broiler chickens fed humic acid supplemented diets for 0 – 21 days

Parameters	T1	T2	T3	SEM	*P*-value
Villus Height (µm)	802.5 ± 144.93^{a}	729.37 ± 152.54^{ab}	621.43 ± 91.83^{b}	59.85	0.018
Villus Width (µm)	123.02 ± 36.54	101.17 ± 9.47	115.31 ± 28.06	12.14	0.459
Crypt Depth (µm)	70.39 ± 16.52	79.77 ± 14.04	71.37 ± 9.21	6.08	0.508
Crypt Width (µm)	33.96 ± 3.06	36.11 ± 4.58	33.21 ± 9.01	2.73	0.743
Villus Height:crypt depth ratio	1.03 ± 0.29^{a}	0.70 ± 0.12^{b}	0.69 ± 0.15^{b}	0.09	0.031
Epithelial thickness (µm)	31.92 ± 10.15	32.30 ± 3.02	32.93 ± 5.23	3.05	0.973
Goblet cell count (µm)	1.20 ± 0.25^{b}	1.67 ± 0.53^{a}	1.06 ± 0.48^{b}	0.20	0.013

Means within the same row with the same superscript are not significantly different ($p > 0.05$). T1 – Control (basal diet); T2 – basal diet + antibiotic fed for 21 days; T3 – basal diet + humic acid fed for 21 day. SEM – Standard Error of Means.

humic acid fed for 21days and humic acid fed for 42 days, respectively. Similar trend was observed in the weight gain of the birds on dietary treatments.

The birds fed antibiotic supplemented diet for 42 days consumed more feed (5021.90 g per bird) when compared with birds placed on antibiotics for 21 days (4651.43 g per bird). However, the feed intake of birds fed the control diet and humic acid diets (0 –21 and 0 – 42 days) were similar.

The highest cost of feed per kg was recorded in those supplemented with humic acid for 42 days (₦ 218.83), while the least (₦ 191.78) was recorded in those of the control group. A resembling pattern was observed in feed cost per kg per bird. The cost of feed per kg weight gain of those on humic acid supplemented diets for 21 days (₦ 94.18) was similar to those in other treatment groups.

Effect of humic acid supplementation on gut morphology of broiler chickens (starter phase)

The results of the gut morphological indices of broiler chickens placed on humic acid supplemented diets are presented in Table 5. There were no significant differences observed in the villus width, crypt depth, crypt width and epithelial thickness of the birds. However, the birds in the control group recorded a villus height (802.5 µm) significantly different from those on humic acid supplemented diet (621.43 µm). A similar trend was also observed in the villus height to crypt depth ratio. The birds on antibiotic supplemented diets recorded a statistically ($p < 0.05$) higher goblet cell count than those in the control and humic acid supplemented diets, respectively.

Effect of humic acid supplementation on gut morphology of broiler chickens 0 – 42 days (finisher phase)

The results of the gut morphological indices of broiler chickens placed on humic acid supplemented diets at 0 – 42 days are as presented in Table 6. There were no significant differences observed in the crypt depth, epithelial thickness and goblet cell count of the birds.

Improved ($p < 0.05$) villus height (724.99 µm), villus width (68.31 µm), crypt width (29.20 µm) and villus height to crypt depth ratio (0.79 µm) were recorded in birds placed on humic acid supplemented diet, which was similar to those on the control diet.

Table 6. Gut morphological indices of broiler chickens placed on humic acid supplemented diets for 0 – 42 days

Parameters	T1	T2	T3	SEM	*P*-value
Villus Height (µm)	626.54 ± 86.72^{ab}	556.77 ± 91.66^{b}	724.99 ± 116.83^{a}	44.70	0.038
Villus Width (µm)	67.67 ± 7.58^{a}	46.76 ± 13.75^{b}	68.31 ± 23.59^{a}	7.32	0.010
Crypt Depth (µm)	80.66 ± 17.13	74.32 ± 15.34	80.01 ± 9.55	6.43	0.751
Crypt Width (µm)	22.17 ± 5.29^{ab}	18.09 ± 3.40^{b}	29.20 ± 7.10^{a}	2.45	0.023
Villus Height:crypt depth ratio	0.69 ± 0.08^{ab}	0.60 ± 0.06^{b}	0.79 ± 0.11^{a}	0.04	0.017
Epithelial thickness (µm)	26.30 ± 6.36	22.84 ± 4.44	27.67 ± 6.29	2.58	0.421
Goblet cell count (µm)	0.83 ± 0.23	0.92 ± 0.27	0.75 ± 0.43	0.14	0.703

Means within the same row with the same superscript are not significantly different ($p > 0.05$). T1 – Control (basal diet); T2 – basal diet + antibiotic fed for 42 days; T3 – basal diet + humic acid fed for 42 day. SEM – Standard Error of Means.

Table 7. Intestinal microbial load of broiler chickens fed experimental diet (×10^3 cfu.mL^{-1})

Parameters	T1	T2	T3	SEM	*P*-value
Total Bacteria Count	22.67 ± 2.66^{a}	4.57 ± 0.06^{c}	7.77 ± 1.12^{b}	0.71	0.0001
Total *E. Coli* Count	12.62 ± 7.09^{a}	2.72 ± 2.82^{b}	0.50 ± 0.08^{b}	2.12	0.0069
Total Coliform Count	20.22 ± 3.21^{a}	3.50 ± 0.69^{b}	4.75 ± 2.23^{b}	1.08	0.0011

Means within the same row with the same superscript are not significantly different ($p > 0.05$). T1 – Control (basal diet); T2 – basal diet + antibiotic fed for 42 days; T3 – basal diet + humic acid fed for 42 day. SEM – Standard Error of Means.

Humic acid supplementation on intestinal microbial population of broiler chickens

The results of the effects of humic acid supplemented diet on intestinal microbial population are presented in Table 7. There were significant ($P < 0.05$) differences across the treatments. Highest total bacteria count was recorded in birds on the control diet, while the least was observed in those placed on antibiotic supplemented diet. Similarly, total *E. coli* and total coliform counts were higher in birds on the control diets compared with those on the antibiotic and humic acid supplemented diets.

DISCUSSION

There are quite a number of reports regarding the beneficial effects of using humic acid in poultry production either supplemented in feed (Eren *et al.*, 2000; Ozturk *et al.*, 2012) or in drinking water at various concentrations (Ozturk *et al.*, 2010). The results of this study show that the inclusion of humic acid to the corn-soya based diets did not increase the final weight and weight gain of the birds at the end of the first 21 days of the feeding trial, but with improved weight gain of the birds at the end of the 42nd day (finisher phase) of the experiment. This agrees with the findings of Karaoglu *et al.* (2004) and Kaya and Tuncer (2009), who reported that including humic acid in the diet of broiler chickens did not affect their body weight and weight gain. Also, Kocabagli *et al.* (2002) noted that adding humic acid into broiler diet at the level ranging from 1.0 to 2.5 g.kg-^{1} feed from 1 – 42 days did not significantly affect their body weight up to the 21st day of age. However, they reported the most beneficial effect in respect to growth by increasing body weight during the growing period determined from 22 to 42 days of age compared with those fed control diet. On the contrary, Eren *et al.* (2000) noted that adding humic acid at the level of 2.5g kg^{-1} feed increased significantly body weight and the body weight gain at 42nd day of age without significant effect at 21st day of age. Ceylan *et al.* (2003) reported that adding humic acid at the level of 5.0 g kg^{-1} broiler diet enhanced body weight gain. TeraVita (2004) indicated that adding dietary humic acid during late period for broiler increased body weight by 30 %. In another study, Lala *et al.* (2017) reported that final weight and weight gain improved with humic acid supplementation in drinking water, which also is not congruent with the findings of this study. This could be attributed to the route of administration, because whilst this study administered the humic acid via the feed, Lala *et al.* (2017) administered humic acid via drinking water.

The results of the present study show improved feed conversion ratio (FCR) of birds at the starter phase. In contrary to the findings of this study that significant differences in the FCR of the birds at the end of the first 21 days of age, and no significant difference in the later age of the birds, Seyed *et al.* (2012) reported that adding 0.1 % – 0.3 % humic acid into the diet improved FCR at different phases of growth of the broiler chickens. Esenbuga *et al.* (2008) reported that the FCR for broiler chicks fed a diet containing humic acid at the level of 1.0 g.kg^{-1} feed was considerably higher by 2.0 per cent than that fed control diet, but the FCR was not affected by humic acid supplementation up to 3.0 g.kg^{-1} broiler diet. Ozturk *et al.* (2010) also reported a lower FCR when humic acid was applied to the drinking water of broiler chickens.

The results obtained in this study regarding the cumulative feed intake (0 – 42days), which was almost similar across the treatments, agree with the findings of Lala *et al.* (2017), who reported no significant difference observed in feed intake upon inclusion of humic acid in drinking water. Despite variations in the routes of administration of the humic acid as well as the concentration of the humic acid administered, the observations were not different. While Lala *et al.* (2017) administered humic acid via drinking water at a maximum concentration of 2 ml.L^{-1} of water, this study administered humic acid via the diet at the concentration of 1 g.kg^{-1} of the diet. At day 42 of the experiment, broiler chickens fed with diets containing humic acid had higher feed cost per kg weight gain but similar to other treatments at day 21. The result of this study disagrees with the findings of Araujo *et al.* (2019), who reported that broilers receiving diets containing antibiotics had higher costs, revenue and profits, which the author traced mainly to higher feed intake, which resulted in higher body weight of birds. Contrary to the findings of this study, Chowdhury *et al.* (2009) reported lowest production cost in broilers found in those fed with organic acids, followed by antibiotics, negative control (without additives) and the interaction among them. Disparities in results could be attributed to prevailing feed costs of feed ingredients and additives at the time and season of the experiment.

At the end of the 21 days of the experiment (starter phase), the villus height and villus height:crypt depth ratio of the birds in the control group was higher than in the birds placed on humic acid supplemented diet, but with continuous feeding of humic acid-based diet up to 42 days, there was an appreciable increase in the villus height, villus width and crypt width of the birds placed on humic acid supplemented diet, however, comparable with those on the control group. The finding of this study agrees with the reports of Seyed *et al.* (2012), who reported that 0.1 – 0.3 % humic acid in the diet increased villus height and crypt depth of the ileum. Longer villus height could be considered as an indication of successful intestinal villi functioning and provides more surface area for nutrient absorption (Shalaei *et al.*, 2014). Acidification decreased intestinal colonization and infectious processes, thus minimizing the inflammatory process of intestinal mucosa, which elevates the height of the villus and its role of secretion, digestion and absorption of nutrients (Iji and Tivey, 1998). The crypt depth recorded in the study as well as the villus height indicates a stable health state as the values obtained fall within the range for a healthy bird, as reported by De Verdal *et al.* (2010). This is possibly due to the ability of humic acids to form protective film on the walls of the intestine, thereby reducing and hindering the activities of harmful bacteria, which affect the intestinal morphology of the birds.

Inclusion of organic acids into poultry diet can inhibit the growth of enteric pathogens, reduce the incidence of disease and promote growth of the birds. Highest total bacteria count was recorded in birds on the control diet, while the least was observed in those placed on antibiotic supplemented diet. Similarly, total coliform count and total *E. coli* count were higher in birds on the control diets compared with those on antibiotic and humic acid diets. The inhibition of the growth of pathogenic bacteria in the GIT can be traced to the buffering capacity of humic acid in the gut thereby modulating the gut pH, as reported by Rath *et al.* (2005) and Arpášová *et al.* (2016). As also observed in the present study, humic acid inhibits pathogenic bacteria growth as well as reduces growth of *E. coli* and coliform, thus affirming the reports of Van Rensburg et al, (2006). Similarly, Schepetkin *et al.* (2003), reported that humate has tremendous ability to alter the intestinal microflora by increasing the counts of beneficial bacteria. The present study is consistent with the reports of Akyurek *et al.* (2011) and Agboola *et al.* (2015) that broiler chickens fed diets containing organic acid blends had less pathogenic bacteria loads, such as coliforms and Clostridia, but greater beneficial bacteria, such as Lactobacilli, in the ileum compared with those fed diets containing antibiotics. Thirumeignanam *et al.* (2006) reported a decrease in total bacterial load with increase in Lactobacilli load because of dietary acidification.

CONCLUSION

This study demonstrates the effects of humic acid supplementation on the growth response and gut integrity of broiler chickens. The results show that humic acid supplemented in the diet of broiler chickens at the level of 1 $g.kg^{-1}$ of feed for 21 days and for 42 days respectively did not have any deleterious effect on the growth performance of broiler chickens at both stages of their growth. The microbial and gut morphological parameters, measured in this study, also indicated improved gut health of the birds.

The study has shown that including 1 $g.kg^{-1}$ of dietary humic acid into the diet of broiler chickens is a viable and possible alternative to antibiotics used as growth promoters in the poultry industry for improved performance and gut integrity.

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