ISSN 0189-0514
J. Anim. Prod. Res. (2017) 29(1): 288-294

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RESPONSE OF BROILER CHICKENS TO VARYING DOSAGE OF HUMIC ACID IN DRINKING WATER

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ABSTRACT

This experiment was conducted to determine the effect of varying dosage of humic acid in the drinking water of broiler chickens. A total of 120 one-day-old Arbor Acre broiler chickens with an average weight of 35g per chick were randomly allotted to three treatment groups of 0, 1 and 2 mL/L humic acid in a completely randomized design. Each treatment group contained 40 chickens with four replicates. Results of the experiment indicated that humic acid ($P<0.05$) increased weight gained, weight gain ($P<0.05$) increased as the level of humic acid increased in the drinking water. Feed consumption of broiler chickens supplemented with humic acid was comparable to feed intake of birds in the control group. Thus, improved feed conversion of birds supplemented with humic acid. Humic acid ($P<0.05$) increased digestive enzyme activities in the pancreas and improved villus height in the small intestine. The study revealed that broiler chickens could tolerate humic acid in the drinking water up to 2 mL/L for improved growth, better nutrient utilization and feed conversion.

Keywords: growth, intestinal morphology, digestive enzymes, Abor Acre chickens

INTRODUCTION

The gastrointestinal tract of newly hatched chicks is immature and sterile. The chick is very susceptible to pathogenic microorganisms until its gut microflora is developed, when it starts to ingest feed (Adams, 2004). Anti-microbial feed additives such as antibiotics are often used to suppress or eliminate harmful organisms in the intestine, and to improve growth and feed efficiency (Jin *et al*., 1997). The benefits of antibiotic growth promoters on the health and growth of animals has been well documented in the scientific literature, as well as the arguments against the use of antibiotic growth promoters that could lead to the development of resistant pathogenic bacteria, which represents a serious health risk to animals and man (Jan *et al*., 2012).

As a result, many alternatives have been used including probiotics, prebiotics, herbal compounds and organic acids. Most of these substances exert their effects by influencing gastrointestinal flora and digestion processes. Among these alternatives the influence of humic acid has been reported to inhibit bacterial and fungal growth, thus decrease levels of mycotoxins in feed (Riede *et al*., 1991).

Humic acid shows antibacterial, antiviral, antithyroideal and antimicrobial effects in animal husbandry to improve the economy and ecology of animal production by increasing growth rate, decreasing feed expenditure per gain and diminishing the risk of disease (Eren *et al*., 2000; Kocabagli *et al*., 2002). Humic acid is known to be nontoxic and nonteratogenic and has been used as analgesic and antimicrobial agent in veterinary practices (Yasar *et al*.,

2002). The present study was carried out to evaluate the effect of humic acid on growth performance, digestive enzymes and morphology of broiler chickens

MATERIALS AND METHODS

Test supplement

Humic acid used is a product of Dynapharmlab Associate SDN. BHD. It contains chelated micronutrients, nitrogen 2.35%, phosphorus 4.44%, potassium 1.75%, magnesium 0.36%, iron 867ppm, manganese 223ppm, copper 144ppm, zinc 153ppm, boron 0.011%, molybdenum 0.002% and humic acid 0.68%.

Experimental birds

A total of 120 one day-old Abor Acre broiler chickens were randomly allocated into 3 treatment groups: 1) control group with no humic acid supplementation, 2) supplementation with 1mL/L humic acid and 3) 2mL/L humic acid supplementation in drinking water. Each treatment group was replicated four times with 10 chicks per replicate. The duration of experiment was 8 weeks; broiler starter mash was fed for the first four weeks while broiler finisher mash was given at the last four weeks as shown in Table 1. Drinking water was refreshed every day.

Table1: Percentage composition of broiler chickens basal diets

Ingredient	Starter (0-4 weeks)	Finisher (4-8 weeks)
Maize	52.90	60.00
Fish meal	2.50	0.00
Soya bean meal	36.60	28.00
Wheat offal	4.00	7.70
Bone meal	1.30	1.80
Oyster shell	1.80	1.50
Premix	0.30	0.30
Salt (NaCl)	0.30	0.30
L-lysine	0.10	0.10
L-methionine	0.20	0.30
Analyzed values		
Crude protein (%)	23.64	19.91
Ether extract (%)	3.61	3.51
Crude fibre (%)	3.22	3.27
Calculated values		
Calcium (%)	1.08	1.09
Phosphorus (%)	0.43	0.39
Metabolizable energy (MJ/kg)	11.98	12.00

Starter premix provided: Vitamin A-10,000,000iu, Vitamin D3-2,000iu, Vitamin E-40,000mg, Vitamin K-2,000mg, Vitamin B1-1,500mg, Vitamin B2-4,000mg, Vitamin B6-40,000mg, Vitamin B12-20mg, Niacin-40,00mg, Pantothenic-10,000mg, Folic-1,000mg, Biotin-100mg, Choline-300,000mg, Manganese-80,000mg, Zinc-60,000mg, Iron-40,000mg, Copper-80,000mg, Iodine-800mg, Selenium-200mg, Cobalt-300mg, Antioxidant-100,000mg.

Finisher premix provided: Vitamin A-12,000,000iu, Vitamin D3-2,500,000iu, Vitamin E-30,000mg, Vitamin K-2,000mg, Vitamin B1-2,250mg, Vitamin B2-6,000mg, Vitamin B6-4,500mg, Vitamin B12-15mg, Niacin-40,00mg, Pantothenic-15,000mg, Folic-1,500mg, Biotin-50mg, Choline-300,000mg, Manganese-80,000mg, Zinc-50,000mg, Iron-20,000mg, Copper-5,000mg, Iodine-1,000mg, Selenium-200mg, Cobalt-500mg, Antioxidant-125,000mg.

Growth performance

Birds were weighed per replicate at the beginning and subsequently on weekly basis to calculate the weight gain. Feed intake was recorded daily and calculated as g per bird; feed conversion ratio was calculated by dividing total feed intake by total weight gain. Mortality was recorded as it occurred in percentage, as the number of dead birds divided by the number of birds stocked multiply by 100.

Digestive enzyme activities of the pancreas

The pancreas sample from one bird per replicate was harvested, frozen, thawed and homogenized in 4 volumes of ice-cold 0.9% NaCl. The homogenate was centrifuged (13,800 × *g* for 20 min at 4°C), and the supernatant were analyzed for digestive enzymes activities. Amylase (a-1, 4-glucan 4-glucanohydrolase, EC 3.2.1.1) activity was determined using the method of Somogyi (1960). Amylase activity unit was defined as the amount of amylase that will cause formation of reducing power equivalent to 1 mg of glucose in 1 h. The substrate used in the analysis was corn starch. Lipase (triacylglycerol lipase EC 3.1.1.3.) activity was assayed using the method described by Tietz and Fiereck (1966). Lipase activity unit was equal to the volume (ml) of 0.05 M NaOH required to neutralize the fatty acid liberated during 2 hr incubation. Olive oil was used as the substrate in this assay. The substrate used for trypsin determination was Nα-Benzoyl-dl-arginine 4-nitroanilide hydrochloride (B4875, Sigma, St. Louis, MO). One unit of trypsin activity was defined as the hydrolysis of 1 μmol of substrate per minute.

Intestinal morphology measurement

Parts of small intestine segments from the duodenum, jejunum, and ileum were collected during slaughter. Sections were flushed with physiological saline and fixed in 10% formalin; three cross-sections for each intestinal sample were stained with hematoxylin and eosin using standard paraffin embedding procedures. Villus height was measured by ocular micrometer under 10x objective of microscope

Statistical Analysis

Data generated were subjected to analysis of variance using the general linear model procedure of the SAS (2000) to determine the effect of humic acid. A probability of ($P<0.05$) was considered to be statistically significant using Duncan's Multiple Range Test of the same package. Polynomial contrast (linear and quadratic) was used to determine the effect of varying dosage of humic acid.

RESULTS

Growth response of broiler chickens supplemented with humic acid in the drinking water is represented in Table 2. Final weight and weight gained showed significant difference among all experimental groups, final weight and weight gained ($P<0.05$) improved with humic acid supplementation. As humic acid increased in the drinking water, final weight and weight gain of the broiler chickens increased. Feed intake and percentage mortality did not indicate any significant ($P>0.05$) difference among the treatment groups. Feed conversion ratio (FCR) improved with humic acid in the drinking water of the broiler chickens.

Table 2: Performance characteristics of broiler chickens supplemented with humic acid

	Humic acid supplemented level (mL/L)				Trend (P-value)	
Parameter	0	1	2	SEM	Linear	Quadratic
Initial weight (g/bird)	35.00	35.00	35.00	0.00		
Final weight (g/bird)	1983.79[b]	2151.88[a]	2198.49[a]	34.71	0.001	0.002
Weight gain (g/bird)	1948.79[b]	2116.88[a]	2163.49[a]	34.71	0.001	0.002
Daily weight gain (g/bird)	34.80[b]	37.80[a]	38.63[a]	0.63	0.001	0.002
Feed intake (g/bird)	4825.03	4795.13	4755.07	23.55	0.250	0.541
Feed conversion ratio	2.48[b]	2.27[a]	2.20[a]	0.05	0.004	0.011
Mortality (%)	6.67	3.33	0.00	1.67	0.104	0.296

[ab] Means with different superscripts within the same row are significantly different (P<0.05)
SEM= standard error of mean

There was significant difference (P<0.05) (Table 3) in the amylase activity of the pancreas among the digestive enzymes measured. The amylase activity of broiler chickens supplemented with humic acid increased in the pancreas when compared with the control group. Pancreatic trypsin and lipase activities were comparatively lower in the control group compared to the group supplemented with humic acid, however, these numerical differences showed no significant difference (P>0.05) in the trypsin and lipase activities. It was observed that humic acid significantly improved villus height in the duodenum, jejunum and ileum. Villus height was significantly (P<0.05) increased by humic acid compared with the control group.

Table 3: Pancreas digestive enzymes and intestinal villus height of broiler chickens supplemented with humic acid

	Humic acid supplemented level (mL/L)				Trend (P-value)	
Parameter	0	1	2	SEM	Linear	Quadratic
Digestive enzymes (Units)						
Amylase	32.87[b]	43.27[a]	41.60[a]	1.72	0.025	0.002
Trypsin	12.33	14.63	14.10	0.48	0.137	0.102
Lipase	0.73	0.87	0.97	0.11	0.437	0.756
Villus height (µm)						
Duodenum	726.67[b]	810.00[a]	813.33[a]	0.09	0.028	0.036
Jejunum	873.33[c]	1003.33[b]	1050.00[a]	0.04	0.043	0.124
Ileum	660.00[c]	713.33[b]	753.33[a]	0.05	0.012	0.056

[ab] Means with different superscripts within the same row are significantly different (P<0.05)
SEM= standard error of mean

DISCUSSION

The positive effect of humic acid on the broiler chickens final weight and weight gain might be attributed to the digestive tract ecosystems. Acidification with various organic acids has been reported to reduce the production of toxic components by bacteria and colonization of pathogens on the intestinal wall, thus preventing the damage to epithelial cells (Langhout, 2000). The ability to create protective layers over the epithelial mucosal membrane of the digestive tract against the penetration of toxic and other bacterial contaminated substances improved utilization of nutrients in animal feed (Islam *et al*., 2005). Ceylan and Ciftci, (2002) reported that humates might increase the uptake of nitrogen, phosphorus, and other nutrients due to their chelating properties. The acid anion has been shown to complex with Ca, P, Mg, and Zn, which results in an improved digestibility of these minerals and serve as substrates in intermediary metabolism (Kishi *et al*., 1999). It has been shown that the addition of a commercial product as a source of humic substances through the drinking water improved the feed intake, weight gain, feed efficiency and carcass weight of broiler chickens (Eren *et al*., 2000; Yasar *et al*. 2002; Ozturk and Coskun, 2006; Ozturk *et al*., 2010).

Lower feed conversion reported in this study could be as a result of improved digestibility of feed consumed, since similar feed were consumed by birds in all treatment groups. Rath *et al*. (2006) reported that humic acid have the ability to increase metabolism of protein and microbial carbohydrates based on catalyzing agents by bringing down the pH of small intestine. Lower feed conversion ratio were reported by Kocabagli *et al*. (2002) and Celik *et al*. (2008) in broiler chickens fed with a commercial product containing humic acid. Also, Ozturk *et al*. (2010) reported lower feed conversion ratio as observed in this study in which humic acid was supplemented in the drinking water of broiler chickens.

The improvement of the pancreas digestive enzyme activities with humic acid supplementation could be attributed to the trace minerals that form part of the humic acid composition. Altering of the pH and the trace elements in the humic acid has been reported to change the enzymatic activity of the gastrointestinal secretions (Martin-Kleiner *et al*., 2001). Humic acid improves protein digestion in young animal by stimulating pancreatic enzyme secretion (Mellor, 2000). Dietary organic acids were reported to suppress the growth of pathogenic bacteria, encourage the growth of beneficial microflora and ensure that the enzymes function at maximal capacity (Broek, 2000; Dibner and Winter, 2002; Ricke, 2003; Dibner, 2004).

Improved intestinal villus height with humic acid supplementation could have increased feed utilization as evidenced by improved weight gain. Longer villus could be considered an indicator of an active functioning intestinal villi and provides more surface area for nutrients absorption (Shalaei *et al*. 2014). Acidification reduced intestinal colonization and infectious process, thereby, decrease inflammatory process at the intestinal mucosa, which improves villus height and function of secretion, digestion and absorption of nutrients (Iji and Tivey, 1998). The use of organic acidifier in the diet significantly increased villus height of different segment of small intestine of broiler chickens possibly by reducing intestinal colonization of pathogenic and non-pathogenic bacteria (Pelicano *et al*., 2005). The increased length of the mucosal villi of the jejunum due to the dietary inclusion of humic substances in broiler have been associated with increased digestibility of nutrients due to a reduction of the passage rate of the intestinal content (Santhin *et al*., 2001; Zhang *et al*., 2005).

CONCLUSION

Supplementation of humic acid up to 2 mL/L in the drinking water of broiler chickens increased digestive enzyme activities and stimulated the villus. This may be responsible for the improved growth performance and better utilization of nutrients by broiler chickens.

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