



## Impacts of dietary humic acid supplementation on growth performance, some blood metabolites and carcass traits of broiler chicks

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### ABSTRACT

This study was designed to evaluate the effect of graded levels of humic acid (HA) supplementation on broiler performance. Day-old broiler chicks (300) were randomly divided into 5 groups under completely randomized design (CRD). Five iso-caloric and iso-nitrogenous broiler starter and finisher diets (HA<sub>0</sub>, HA<sub>1</sub>, HA<sub>2</sub>, HA<sub>3</sub> and HA<sub>4</sub>) containing 0, 0.75, 1.5, 2.25 and 3 g/kg HA, respectively, were formulated and were randomly allotted to each group. Results indicated that, during starter phase highest weight gain (WG) was observed in chicks fed HA<sub>2</sub> diet, but during finisher phase it was observed in birds fed HA<sub>3</sub> diet. Weight gain during whole experiment was also highest in broiler fed HA<sub>3</sub> diet. The best feed conversion ratio (FCR) was also observed in broiler fed HA<sub>3</sub> diets. There were no influences of humic acid on carcass weight, liver weight, heart weight, gut length, breast weight, thigh weight and abdominal fat weight. Blood glucose, urea, Ca, P, creatinine, red blood cells, white blood cells and haemoglobin were not affected by humic acid supplementation but blood cholesterol and low density lipoprotein (LDL) level were significantly reduced ( $P < 0.05$ ) in HA<sub>3</sub> and HA<sub>4</sub> diets. Therefore, it could be concluded that humic acid as an organic feed additive could improve growth performance of broiler chicks and reduce levels of total and low density lipoprotein cholesterol in the blood. The best level of HA to be recommended is 2.25 g/kg diet.

**Key words:** Blood cholesterol, Broiler, Carcass, Growth performance, Humic acid

The use of antibiotics at sub-therapeutic level as growth promoter caused resistant in bacteria which reside in the gut (Phillips *et al.* 2004, Hernandez *et al.* 2006). Due to this adverse situation, the European Union (EU) banned the antibiotics at sub therapeutic level as growth promoters in poultry diet in 2006, since then sub-therapeutic use of antibiotics is not practiced (Castanon 2007, Apatá 2009, Yang *et al.* 2009). Therefore, the poultry nutritionists are trying to substitute the antibiotics with different natural feed additives, such as organic acids and probiotics that can be beneficial similar in control of infectious diseases and ultimately improve feed efficiency (Zhang *et al.* 2005, Alamo *et al.* 2007, Abd El-Hack *et al.* 2015, Alagawany *et al.* 2015a, b, c). By using different feed additives growth

and feed conversion ratio will be improved and also result in disease prevention. Organic acids have been practiced for some decades as feed preservatives for protecting the destruction of feed from microbes and fungus (Paul *et al.* 2007). Acidification of diet has resulted in improved digestibility of protein and absorption of amino acids. It also proved that digestibility and utilization of mineral elements also improved through acidification of diet which ultimately improved the growth performance (Haque *et al.* 2009).

Humic acid (HA) is an organic substance which is acquired by decomposition of organic matter and having long chain molecular formula with high molecular weight. It is insoluble in strong acids having pH below than 2 and soluble in alkaline media (Islam *et al.* 2005). These substances have medium molecular size and their molecular weight is around 5,000 to 100,000 Dalton. Oxygen represents 33–36 %, and nitrogen represents 4 % in this substance. Because of their medium molecular size, sufficient negative surplus charge on their surfaces for peptizing the macromolecules will be present only in a more alkaline medium with a pH over 8. So their mobility in the soil is very limited in neutral acidic-alkaline conditions

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(Khan 2005). Humic acid was used in poultry as growth promoter (Bailey *et al.* 1996, Parks 1998, Shermer *et al.* 1998, Eren *et al.* 2000). Humic acid reduced stress (Enviromate 2002) and enhanced immunity of birds (Enviromate 2002, Humin Tech 2004). Humic acid improved the protein digestibility of animals especially in broilers (Kreutz and Schlikekewey 1992, Huang *et al.* 1994, Seffner *et al.* 1995, Yang *et al.* 1996). Use of HA on daily basis showed positive effect on broilers growth performance (Bailey *et al.* 1996, Parks 1998, Shermer *et al.* 1998, Eren *et al.* 2000, Kocabađli *et al.* 2002, Humin Tech 2004). Humic acid supplementation in broiler could improve body weight and feed efficiency and also enhance fat deposition on thigh muscles (Ozturk *et al.* 2010). Humic acid possessed potential to reduce bacterial and mold growth, by inhibiting the mold growth, toxin level can be reduced (Humin Tech 2004). Limited information is available of humic acid in poultry. Therefore, the following study was planned to investigate the impact of different levels of dietary humic acid supplementation on growth performance and carcass traits of broiler chicks.

#### MATERIALS AND METHODS

**Experimental birds, housing and management:** Day-old broiler chicks (300) with average initial body weight of  $37.83 \pm 1.34$  g were purchased from a local commercial hatchery and at the beginning of first week. Each treatment group was placed in a separate pen ( $4 \times 3 \times 2.5$ ). The total time period of the trial was thirty-five days. Light, ventilation, relative humidity and floor space were maintained as standard. Temperature was kept at 95 °F during first week and then it was decreased as 5 °F/week until it was maintained at 75 °F throughout the trial. This investigation was approved by Department of Animal Sciences College of Agriculture University of Sargodha Pakistan ethical committee.

**Experimental design and diets:** On arrival, chicks were offered with a 250 g/liter sugar solution to provide energy and to overcome the stress of transportation. The chicks were randomly divided into 5 treatment groups of 10 chicks each and then were weighed. The 5 treatments were assigned to the 5 experimental groups ( $T_0$ ,  $T_1$ ,  $T_2$ ,  $T_3$  and  $T_4$ ) randomly in such a way that each treatment was fed to six replicates. Humic acid was added in four dietary treatment groups  $T_1$ ,  $T_2$ ,  $T_3$  and  $T_4$ , respectively at a level of 0, 0.75 g/kg, 1.5 g/kg, 2.25 g/kg and 3.0 g/kg of diet dry matter, while  $T_0$  was treated as a control group. It was further divided into 2 phases; broiler starter phase (1–21 day and broiler finisher phase (22–35 day). The diets were formulated by using excel sheet, the requirement as given by commercial poultry nutrition by Leesson and Summers (2009). An iso-nitrogenous (20% CP), and iso-caloric (2,717 Kcal/kg) starter diet was formulated (Table 1) and fed from day 1 to 21 as given in treatment program. In same way an iso-nitrogenous (19.45 %) and iso-caloric (2,753 Kcal/kg) finisher diet was formulated and fed from day 22 to 35 as given in treatment program. The birds were fed *ad lib.*

Table 1. Ingredients and experimental diets of the starter phase of broiler chickens

Items	Diets	
	Starter (1–21 d)	Finisher (22–35 d)
Ingredient %		
Pigeon pea 20% CP	41.6	45.0
Rice polishing 11% CP	20.0	20.0
Soybean meal 44% CP	8.80	12.30
Canola meal 39% CP	8.10	5.00
Sunflower 28%	5.50	4.50
Poultry meal 82.2% CP	5.00	4.00
Fish meal 50%	4.30	3.10
Salt	4.00	3.00
Premix <sup>1</sup>	1.30	1.30
Limestone	0.30	0.50
Di Calcium phosphate	0.30	0.30
Lysine	0.20	0.30
DL-methionine	0.20	0.20
Nutrient composition, % <sup>2</sup>		
Crude protein	20.00	19.45
ME (kcal/kg)	2717	2753
Lysine	1.30	1.25
Methionine	0.52	0.48
Calcium	1.50	1.10
Available phosphorus	0.40	0.40
Chemical composition, % <sup>3</sup>		
Crude protein	21.11	18.81
Crude fat	5.30	5.30
Crude fiber	5.02	5.11

<sup>1</sup>Supplied per kg of diet: vitamin A, 7500 IU; vitamin D3, 2500 IU; vitamin E, 8 mg; vitamin K3, 2 mg; vitamin B1, 3 mg; vitamin B2, 12 mg; vitamin B6, 4 mg; vitamin B12, 0.03 mg; nicotine amid, 37 mg; calcium-D-pantothenate, 13 mg; folic acid, 0.50 mg; D-biotin, 0.10 mg; choline chloride, 500 mg; Mn, 66 mg; Fe, 27.5 mg; Zn, 55 mg; Cu, 6 mg; I, 1.2 mg; Co, 0.1 mg; Se, 0.15 mg. <sup>2</sup>Calculated according to NRC (1994). <sup>3</sup>Determined according to AOAC (2006).

throughout the experiment. All the chicks were vaccinated against New Castle disease (ND) and infectious bursal disease (IBD) in this trial.

#### Collection of the data

**Growth performance:** Chicks were weekly weighed individually at intervals. Mortality rate was daily recorded (dead/total number $\times$ 100). Average daily feed intake, body weight gain and feed intake were calculated from these data by period and cumulatively. Feed wastage was daily recorded and the data were used to estimate the feed intake.

**Carcass characteristics:** At the termination of the experiment, 30 birds (five each group) were sampled randomly for carcass evaluations at 5 weeks of age, weighed and manually slaughtered. The carcasses were weighed and the weights of the liver, gizzard and heart were recorded and expressed as g/kg of slaughter weight (SW). Carcass and dressed weights were studied [(dressed weight = carcass

weight + giblets weight)/ live body weight].

**Blood sampling and laboratory analyses:** Blood samples were randomly collected from six birds/treatment after slaughtering into sterilized tubes that were closed with rubber stoppers. Blood glucose (mg/dl) was determined using a sample of whole blood. Samples were left to coagulate and centrifuged at 3500 rpm for 15 min to obtain serum, and the serum samples were kept in Eppendorf tubes at  $-20^{\circ}\text{C}$  until analyzed. The following serum biochemical parameters (mg/dl): urea, calcium, phosphorus, total cholesterol, creatinine, high density lipoprotein (HDL) cholesterol, low density lipoprotein (LDL) cholesterol levels were determined spectrophotometrically using commercial diagnostic kits, according to Akiba *et al.* (1982).

**Statistical methods:** The data collected during the research trial on growth performance like feed intake, weight gain, feed conversion ratio and carcass characteristics like dressing percentage, carcass percentage, breast meat yield, thigh meat yield and weight of giblet organs (liver, heart and gizzard) were subjected to statistical analysis using analysis of variance technique under completely randomized design (Steel *et al.* 1997).

## RESULTS AND DISCUSSION

**Effect on growth performance:** A significant impact ( $P < 0.05$ ) on feed intake was observed during finisher or overall by the supplementation of humic acid but no ( $P > 0.05$ ) influence on starter feed intake was observed (Table 2). Results of present study are in agreement with Kemal *et al.* (2008) and Edmonds *et al.* (2014) who concluded that supplementation of humic acid and humic substances in diet resulted in significant improvement in feed intake. It might be due to increase in mass of intestinal epithelial cell leading to improved feed intake. Present findings are contradictory to the findings of Kaya *et al.* (2009), Hakan *et al.* (2012), who reported no significant improvement in

feed intake in poultry birds fed humic acid supplemented diets. No improvement in feed intake might be due to higher energy content in diet or any managerial stress (Ferket and Gernat 2006).

Humic acid had significantly altered starter and finisher weight gain ( $P < 0.05$ ). Highest starter and finisher weight gains were observed in HA<sub>4</sub> and HA<sub>3</sub> groups, respectively. While the lowest weight gain was observed in HA<sub>1</sub> ( $P < 0.05$ ) but highest overall weight gain was observed in birds fed HA<sub>3</sub> (Table 2). Results of present study are in line with the findings of Kemal *et al.* (2008) and Šamudovská and Demeterová (2010) who concluded that the supplementation of dietary humic substances and humic acid showed significant differences on body weight gain of broiler. In another experiment, Avci *et al.* (2007) also found that humic acid in diet improved live body weight. Similarly, Kocabađli *et al.* (2002) reported that the dietary humates supplemented to broiler diets significantly affected live weight gain. Possibly, gut micro flora of animal is stabilized by HA and result in increased nutrient absorption and improved weight gain (Shermer *et al.* 1998, Humin Tech 2004).

The results of present study are not in agreement with the findings of Kaya *et al.* (2009) who reported that there were no differences due to humates supplementation on weight gain in broilers. Celik *et al.* (2007) observed no difference in weight gain by addition of 0.4% organic acid in diet. Also, Bailey *et al.* (1996), Karaoglu *et al.* (2004) reported that the addition of humates and humic acid in broiler diet showed no effect on body weight gain. Humic acid inhibits pathogenic bacterial mould growth and thus improves gut health (Humin Tech 2004). The macro-colloidal structure of HA has a shielding influence on the mucous membrane of the stomach and gut. As a result, the absorption of toxic metabolites is reduced or fully prevented. Humic acids also help to prevent excessive loss of water via the intestine (Humin Tech 2004).

Dietary humic acid supplementation had significant ( $P < 0.05$ ) effect on feed conversion ratio (FCR) during starter and finisher phase (Table 3). During starter phase, FCR of HA<sub>3</sub> and HA<sub>4</sub> diets was better than HA<sub>0</sub>, HA<sub>1</sub> and HA<sub>2</sub> diets. But during finisher phase, FCR of HA<sub>0</sub>, HA<sub>1</sub>, HA<sub>2</sub> and HA<sub>4</sub> diets was higher than that of HA<sub>3</sub> diet. Overall FCR was significantly ( $P < 0.05$ ) improved by humic acid supplementation and HA<sub>3</sub> diet was the best. Our results herein are consistent with the findings of Bailey *et al.* (1996), Avci *et al.* (2007) who postulated the FCR was significantly improved with supplementing varying levels of humates and humic acid in broiler diets. Also, Ceylan *et al.* (2003) performed an experiment to observe the influence of antibiotic growth promoter, probiotics, prebiotics and humic acid fed in corn-soya based diet on growth performance and gut micro flora of broiler chicks; improved feed conversion ratio was observed in a group supplemented with probiotics, prebiotics and humic acid based mixture as compared with control group. In another study using Farmagülatör DRY™ Humates @ 2.5 g/kg, live performance showed better FCR (Kocabađli *et al.* 2002).

Table 2. Effect of humic acid on growth performance in broilers

Items	Diets <sup>1</sup>					SEM <sup>2</sup>
	HA <sub>0</sub>	HA <sub>1</sub>	HA <sub>2</sub>	HA <sub>3</sub>	HA <sub>4</sub>	
Feed intake (g)						
Starter	1049.4	1020.2	1044.0	1014.6	1006.4	12.71
Finisher	2032.2 <sup>a</sup>	1994.2 <sup>ab</sup>	1982.0 <sup>ab</sup>	1973.6 <sup>ab</sup>	1958.0 <sup>b</sup>	15.07
Overall	3081.6 <sup>a</sup>	3014.4 <sup>ab</sup>	3026.0 <sup>ab</sup>	2988.2 <sup>b</sup>	2964.4 <sup>b</sup>	18.59
Weight gain (g)						
Starter	638.60 <sup>b</sup>	656.20 <sup>ab</sup>	674.00 <sup>a</sup>	667.40 <sup>a</sup>	671.40 <sup>a</sup>	5.53
Finisher	922.20 <sup>c</sup>	952.40 <sup>c</sup>	1022.8 <sup>b</sup>	1156.6 <sup>a</sup>	1120.4 <sup>a</sup>	14.84
Total	1560.8 <sup>c</sup>	1608.6 <sup>c</sup>	1696.8 <sup>b</sup>	1824.0 <sup>a</sup>	1791.8 <sup>a</sup>	19.70
Feed conversion ratio (g feed/ g gain)						
Starter	1.64 <sup>a</sup>	1.58 <sup>ab</sup>	1.56 <sup>ab</sup>	1.50 <sup>b</sup>	1.50 <sup>b</sup>	0.03
Finisher	2.22 <sup>a</sup>	2.10 <sup>ab</sup>	1.94 <sup>b</sup>	1.72 <sup>c</sup>	1.76 <sup>c</sup>	0.04
Overall	1.98 <sup>a</sup>	1.88 <sup>ab</sup>	1.78 <sup>b</sup>	1.64 <sup>c</sup>	1.66 <sup>c</sup>	0.03

<sup>1</sup>HA<sub>0</sub>, HA<sub>1</sub>, HA<sub>2</sub>, HA<sub>3</sub> and HA<sub>4</sub> stand for diets having 0, 0.75, 1.5, 2.25 and 3.0 g/kg humic acid. <sup>2</sup>SEM stand for standard error mean. <sup>a,b</sup>In a row means sharing different superscripts differ significantly ( $P < 0.05$ ).

Moreover, Arafat *et al.* (2015) claimed that feed conversion of laying hens was improved ( $P < 0.05$ ) with the administration of humic acid in drinking water. In the present study the mortality rate was nil. Because humic acid could stabilize the intestinal flora and improve utilization of nutrients, the increase in live weight without increasing the amount of feed given to the birds resulted in better FCR (Humin Tech 2004).

Results of present study are in contrast with those reported by Kaya *et al.* (2009) and Celik *et al.* (2007) who reported that dietary humates and other organic acids with probiotics showed no significant improvement in FCR when supplemented in broiler ration. The study is also contradictory to Karaoglu *et al.* (2004) who reported that no significant improvements were observed in FCR in broilers fed humic substances. Furthermore, Ghahri *et al.* (2010) observed an increase in FCR values in broilers fed HA compared to the control group.

**Carcass traits and visceral organs:** Results of the effect of humic acid levels on carcass traits are illustrated in Table 3. Diets with different levels of humic acid supplementation did not alter ( $P > 0.05$ ) carcass weight, breast weight, thigh weight, neck weight and abdominal fat as compared to the basal diet group. The results of present study are in agreement with the findings of Karaoglu *et al.* (2004) and Avci *et al.* (2007) who reported that carcass yield, abdominal fat, neck weight, breast and thigh weight were not significantly improved by supplementation of humic acid and humates in broilers diet. Similar findings were observed by other researchers (Kemal *et al.* 2008, Santos *et al.* 2005, Kaya *et al.* 2009) who used humates, humic acid and several other feed additives (antibiotic, fumaric acid, probiotic, mannan oligosaccharides, mushroom extract and fructo-oligosaccharide) in broiler diet.

**Visceral organs:** No significant impacts ( $P < 0.05$ ) due to humic acid supplementation were observed on gizzard, liver and heart weights and gut length either (Table 4). Our results are supported by Kaya *et al.* (2009) who confirmed that gut length, gizzard, liver and heart weights were not affected by addition of humic acid and humic substances in broiler diet or drinking water. Moreover, the studies of Karaoglu *et al.* (2004), Avci *et al.* (2007), Celik *et al.* (2007) and Kemal *et al.* (2008) observed that gizzard, liver and heart weights were unaltered in birds fed humates in diet.

**Blood metabolites:** As shown in Tables 5 and 6, humic acid supplementation did not affect ( $P > 0.05$ ) blood glucose, urea, Ca, P, and creatinine. Similarly, no significant influence ( $P > 0.05$ ) was observed in red blood cells, white blood cells and haemoglobin. Significant difference was observed in blood cholesterol and high density lipoprotein (HDL). Low density lipoprotein (LDL), the harmful cholesterol, was significantly decreased in humic acid supplemented diets. Lowest LDL values were observed in HA<sub>4</sub> diet. Similarly diets HA<sub>3</sub> and HA<sub>4</sub> showed lowest blood cholesterol values. These results are in harmony with the findings of Avci *et al.* (2007), Kaya *et al.* (2009), Zralý *et al.* (2008), Šamudovská and Demeterová (2010) who

Table 3. Effect of humic acid on carcass traits of broilers

Items (g)	Diets <sup>1</sup>					SEM <sup>2</sup>
	HA <sub>0</sub>	HA <sub>1</sub>	HA <sub>2</sub>	HA <sub>3</sub>	HA <sub>4</sub>	
Carcass weight	1139.0	1140.4	1136.0	1146.4	1144.8	9.54
Breast weight	449.40	469.40	454.40	473.40	450.20	12.1
Neck weight	63.2	63.4	65.2	64.2	66.2	1.36
Abdominal fat	32.4	30.6	28.6	33.2	31.0	1.31
Thigh weight	173.4	176.2	173.0	177.40	170.80	3.1

<sup>1</sup>HA<sub>0</sub>, HA<sub>1</sub>, HA<sub>2</sub>, HA<sub>3</sub> and HA<sub>4</sub> stand for diets having 0, 0.75, 1.5, 2.25 and 3.0 g/kg humic acid. <sup>2</sup>SEM stand for standard error mean.

Table 4. Effect of humic acid on visceral organs of broilers

Items	Diets <sup>1</sup>					SEM <sup>2</sup>
	HA <sub>0</sub>	HA <sub>1</sub>	HA <sub>2</sub>	HA <sub>3</sub>	HA <sub>4</sub>	
Gizzard weight(g)	54.6	50.4	51.2	51.6	52.8	1.2
Gut length (cm)	9.04	9.06	9.2	8.96	9.0	0.01
Heart weight (g)	11.8	11.4	12.6	13.8	14.8	1.14
Liver weight (g)	42.6	40.8	42.6	42.4	40.6	1.57

<sup>1</sup>HA<sub>0</sub>, HA<sub>1</sub>, HA<sub>2</sub>, HA<sub>3</sub> and HA<sub>4</sub> stand for diets having 0, 0.75, 1.5, 2.25 and 3.0 g/kg humic acid. <sup>2</sup>SEM stand for standard error mean.

reported that blood Ca, P, glucose, triglyceride and LDL were not affected by humic acid and humic substances in broiler and quails rations. Other researchers (Rath *et al.* 2006, Kemal *et al.* 2008, Hakan *et al.* 2012) also reported similar results when humic acid was supplemented in broiler and layers diets. Moreover, results of current study are in concordant with the findings of Rath *et al.* (2006) who found that red blood cells and white blood cells were not significantly improved by humic acid supplementation in broiler diet. Reduction in blood cholesterol and lipids might be due to decrease in microbial intracellular pH (Abdo and Zeinb 2004). By inhibition of microbial enzymes, bacterial cell membrane is forced to use energy to release acidic protons which lead to decrease in intracellular pH (Young and Foegeding 1993). Red blood cells and haemoglobin level remained similar in control as compared to those fed humic acid supplemented diets but RBCs was capable to carry more oxygen in presence of humate (Lotosh 1991). According to Dabovich *et al.* (2003), HA has a nutraceutical property that stimulated neutrophil activity which might protect against bacterial pathogens and reduce mortality during acute bacterial infection.

Contradictive results were obtained by Kemal *et al.*

(2008) and El-Husseiny *et al.* (2008) who reported that humic acid and humic substances significantly affected blood P, Ca, cholesterol, HDL and LDL. Rath *et al.* (2006) found that blood urea nitrogen, glucose, Ca and P were significantly reduced by HA supplementation. The reduction of blood Ca is due to chelating property of HA having large number of carboxylic acid chain (Klocking *et al.* 1994). As higher molecular weight with long chain molecular formula of HA (Islam *et al.* 2005) it absorbed minerals and other nutrients and made cell membrane more permeable and these nutrients were easily absorbed and overcame metal toxicity (Livens 1991, Hammock *et al.* 2003). Results are also contradictory to the findings of Lotosh (1991) who observed that red blood cells and haemoglobin significantly improved by using humates in broiler diet. It might be due to different age and strains of broiler (Talebi *et al.* 2005). Miçeta *et al.* (2012) postulated that humic substances supplementation to diets of growing rabbits did not change the erythrocytic indices.

In view of the results obtained from this study, it could be concluded that humic acid as an organic feed additive could improve growth performance of broiler chicks and reduce levels of total and low density lipoprotein cholesterol in the blood. The best level of HA to be recommended is 2.25 g/kg diet.

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