

EFFECT OF HUMIC ACID SUPPLEMENTATION ON PRODUCTIVE PERFORMANCE, BLOOD CONSTITUENTS, IMMUNE RESPONSE AND CARCASS CHARACTERISTICS OF SASSO CHICKEN

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SUMMARY

Unsexed seven-day-old Sasso chicks were used to determine the effect of humic acid on productive performance, blood parameters, carcass characteristics, immune response and economical efficiency of Sasso chicks. At seven days of age 196 birds were distributed into four groups (49 birds in seven replicates). The chicks were fed basal diet and were submitted to the following dietary treatments: the first group fed a basal diets without supplementation (control), the 2nd, 3rd and 4th groups fed the same basal diets supplemented with 0.1, 0.2 and 0.4% of humic acid. At the end of the experiment, some carcass characteristics were measured and blood samples were taken to determine some blood plasma constituents. The results reported that Sasso chicks fed 0.1% of humic acid had greater productive performance and economical efficiency than those fed basal diet (control). Chicks fed 0.1% of humic acid had significantly higher glucose than the control group. Supplementation of humic acid decreased serum AST, ALT, urea, creatinine, total lipids, triglycerides, cholesterol, HDL, LDL, MCV, MCH, α -globulin and β -globulin and increased T4, GPX, SOD, RBC's hemoglobin, WBC's, total protein, α -globulin, LA, BA, LTT, phagocytic activity and phagocytic index compared to control group (within normal range). Feeding diet with 0.1 and 0.2% of humic acid significantly increased the percentage of dressing and decreased abdominal fat compared to control. Moreover, humic acid decreased bacterial count of the digestive tract compared to control group. In conclusion, humic acid supplementation at 0.1, 0.2 and 0.4% improved growth performance, nutrient digestibility, production index and economical efficiency especially at the level of 0.1%, without any adverse effects on blood components of Sasso chicken.

Keywords: Sasso chicks, humic acid, productive performance, blood parameters, economical efficiency

INTRODUCTION

Bacteria that resides in the intestines showed resistant to antibiotics used as growth promoter (Hernandez *et al.*, 2006). So, the European Union (EU) banned using the antibiotics as growth promoters in poultry diet in 2006, since the sub-therapeutic use of antibiotics is not practiced (Yang *et al.*, 2009). Humic acids, one of the potential substances alternatives to antibiotics in the diet of poultry (Nagaraju *et al.*, 2014), are naturally produced from the decomposed organic constituents of soil and lignite (MacCarthy, 2001).

Lately, Humic acids has been used to promote growth for poultry in the feed and water (Rath *et al.*, 2006 and Arif *et al.*, 2016). Salah *et al.* (2015) and Arif *et al.* (2016) indicated that the supplementation of humic acid had significantly improved body weight gain and FCR of broiler. Ozturk *et al.* (2014) and Nagaraju *et al.* (2014) found that addition of humic acids in the broiler diets improves meat quality, weight gains and the immune system. Ozturk *et al.* (2010) reported that humic acids supplementation improves growth, meat quality, carcass characteristics as well as, parameters determined in the blood and in the gastrointestinal tract.

Broiler chickens supplemented with humic acid showed significant increases in leukocytic count, lymphocyte, phagocytosis, phagocytic index, total proteins α , β , and γ globulin, coupled with significant decreases in heterophils, monocyte, aspartate aminotransferase, alanine aminotransferase, Alkaline phosphates, A/G ratio beside increase decrease of serum total protein, albumin, uric acid, creatinine and insignificant in eosinophilia and basophilia (Salah *et al.*, 2015). The aim of this study was to determine the effect of humic acid on productive performance, blood parameters, carcass characteristic and immune response of Sasso chicks from day 7 to 51 of age.

MATERIALS AND METHODS

This study was conducted at the Poultry Research Unit (El-Bostan Farm), Department of Animal and Poultry Production, Faculty of Agriculture, Damanhour University, Damanhour, Egypt, from May to June, 2016.

One hundred and ninety six unsexed seven-days-old Sasso chicks obtained from a commercial hatchery (127.6 \pm 0.84 gm) were randomly distributed into four groups (n=49 birds), each group contain seven replicates (7 birds per replicate) and reared on

similar managerial conditions. The chicks were fed basal diet and were submitted to the following dietary treatments: the first group fed a commercial basal diets without supplementation (control), the 2nd, 3rd and 4th groups fed the same basal diets supplemented with 0.1, 0.2 and 0.4% of humic acid. Humic acid (powder) was obtained from Humin Tech, Germany

(purchased by Growtech, Agent in Egypt). The experimental diets were formulated according to NRC (1994). Ingredients and chemical composition of the experimental basal diets (% as fed basis) fed during the three phases (starter from d 7 to 20, grower from d 21 to 34 and finisher from d 35 to 51 day) are shown in Table (1).

Table 1. Ingredients and chemical composition of the experimental basal diets

Ingredients (%)	Starter	Grower	Finisher
Yellow corn	53.70	53.90	60.80
Wheat bran	6.45	12.00	7.00
Soybean meal (44% CP)	23.50	18.00	17.30
Vegetable oil	2.50	5.00	5.00
Gluten meal	10.00	7.32	6.00
Dicalcium phosphate	1.80	1.70	1.80
Limestone	1.00	1.00	1.00
L-Lysine	0.30	0.30	0.30
DL-Methionine	0.15	0.18	0.20
Vit+min premix ¹	0.3	0.30	0.30
NaCl	0.3	0.30	0.30
Total	100	100	100
Calculated and determined composition,			
ME (Cal/kg) ³	3016	3116	3211
CP, % ³	22.45	19.24	18.07
CP, % ²	22.07	19.10	18.20
Lysine, % ³	1.13	1.07	0.98
Methionine, % ³	0.53	0.51	0.51
Meth+cystine, % ³	0.91	0.83	0.80
Calcium, % ³	0.93	0.91	0.91
Av. P, % ³	0.44	0.43	0.41
Crude Fat, % ²	6.04	6.38	6.78
Crude fibre, % ²	3.45	3.78	3.65
Ash, % ²	5.10	5.34	5.68

¹Vit+Min mix. provides per kilogram of the diet: Vit. A, 12000 IU, vit. E (dl- α -tocopheryl acetate) 20 mg, menadione 2.3 mg, Vit. D3, 2200 ICU, riboflavin 5.5 mg, calcium pantothenate 12 mg, nicotinic acid 50 mg, Choline 250 mg, vit. B₁₂ 10 μ g, vit. B₆ 3 mg, thiamine 3 mg, folic acid 1 mg, d-biotin 0.05 mg. Trace mineral (mg/ kg of diet): Mn 80 Zn 60, Fe 35, Cu 8, and Selenium 0.1 mg. ²Analyzed values. ³Calculated values.

Chicks were housed in wire cages (60 cm Length \times 50 cm depth \times 40 cm Height) provided with galvanized feeders and automatic nipple drinkers in semi-opened room equipped with two exhaust fans to keep normal ventilation. Chicks were fed the experimental diets *ad libitum* and given free access to water. A light schedule similar to commercial conditions was applied until 7th day being 23 h light followed by 20 h light from 8th day until 3 days before slaughter test (8-48 days of age). The average outdoor minimum and maximum temperature and relative humidity during the experimental period was 22C^o and 24 C^o and 55.7 % and 58.7%, respectively. The brooding temperature (indoor) was 32, 30, 27 and 24-21 C^o during 1-7, 8-14, 15-20 and 21-51 days of age (declined gradually).

Chicks in each replicate were weighed (g) at 7, 28 and 51 days of age, and the BWG (g/chick) was calculated. Feed intake was recorded for each replicate (g/chick) and thereby FCR (g feed/g gain) was calculated. Production index value was calculated throughout the experimental period (7-51d) of age (Attia *et al.*, 2012) as below.

$$PI = \frac{BW (kg) \times SR}{PP \times FCR} \times 100$$

Where:

EPEI = European Production Efficiency Index

BW = Body weight (kg)

SR= Survival rate (100% - mortality)

PP= Production period (days)

FCR = Feed conversion ratio (kg feed / kg gain)

At the 51 days of age the apparent digestibility of nutrients and ash retention was done using five birds per treatment housed individually in metabolic cages/treatment using total collection method as cited by (Abou-Raya and Galal, 1971). Nitrogen, EE, CF and ash content of the dried excreta as well as those of feed were determined according to (AOAC, 2004). Economical evaluation for all experimental treatments was made (Zeweil, 1996) as below.

$$\text{Economic efficiency} = \frac{\text{Total revenue} - \text{Total cost}}{\text{Total cost}} \times 100$$

Where:

Total revenue = BW \times Meat Price

Total cost = Feed cost + Addition cost + Other cost

At 51 d of age, five chicks were taken randomly from each treatment and slaughtered to determine dressing percentage.

Five blood samples (about 3 ml) were collected before slaughter from the wing vein for hemato-biochemical analysis. Heparin was used as anticoagulation, but a portion of each sample was taken without heparin to obtain serum. Plasma or serum were separated by centrifugation of the blood at 3000 rpm for 20 minutes and stored at -20°C for later analysis. Biochemical indicators such as (Glucose, Urea, Creatinine, ALT, AST(U/L), Alkaline phosphatase, Total Lipid, Triglycerides, Cholesterol, HDL, LDL, Total antioxidant capacity (TAC), glutathione peroxidase (GPX), glutathione (GSH), superoxide dismutase (SOD), T3, T4), Hematological traits such as (RBC's, Hemoglobin, PCV, MCV, MCH, MCHC, WBC's, Lymphocytes, Monocytes, Basophils, Eosinophils, Heterophiles) and Immune indices such as (Total protein, Albumin, Globulin, α -globulin, globulin β , Globulin γ , Lysozyme activity (LA), Bactericidal activity (BA), Lymphocyte transformation test (LTT), Phagocytic index (PI), Phagocytic activity (PA), immunoglobulins (IgY, IgM and IgA) were measured as described previously by (ELnaggar *et al.*, 2016).

At the time of slaughter, 5 samples of cecal content for each treatment were taken for bacterial counting. The effect of dietary treatments on the microbial activity of the digestive system include: total bacteria count which was determined according to the method of (ICMSF, 1980), as well as the detection of *Salmonella* and *Escherichia coli* strains following the ISO-6579: 2002 food microbiology procedure employing the horizontal method of food and animal feeding stuffs (ISO Standards catalogue 07.100.30; WHO 2010).

Finally, samples of breast and thigh meat (50:50 basis) from slaughtered birds and the experimental diets were chemically analyzed according to (AOAC, 2004) and breast and thigh total antioxidant capacity (TAC) was determined by the ORAC assay (Cao and Prior, 1999).

Data obtained were analyzed using the GLM procedure of Statistical Analysis System (SAS, 2002), using one-way ANOVA as in the following model:

$$Y_{ik} = \mu + T_i + e_{ik}$$

Where, Y is the dependent variable; μ is the general mean; T is the effect of experimental treatments; and e is the experimental random error. Before analysis, all percentages were subjected to logarithmic transformation ($\log_{10}x+1$) to normalize data distribution. The differences among means were determined using Duncan's new multiple range test (Duncan, 1955).

RESULTS AND DISCUSSION

Production performance:

Data presented in Table 2 showed the effect of feeding graded levels of humic acid, on body weight,

body weight gain, feed intake, feed conversion ratio, economic efficiency and production index of Sasso strain chicks. Initial body weight (BW) of chicks was similar for all treatments. Sasso chicks fed basal diet supplemented with 0.1% of humic acid had significantly greater ($P \leq 0.01$) body weight at 28 and 51 d of age, body weight gain (from 7-28, 29-51 and 7- 51 days) and production index and better FCR (from 7-28, 29-51 and 7- 51 days) followed by those fed basal diet supplemented with 0.2% then 0.4 % than the control group. Feed intake (from 7-28, 29- 51 and 7- 51 days) was decreased ($P \leq 0.01$) by the inclusion of either level of humic acid compared to control group. Moreover, sasso chicks fed basal diet supplemented with 0.1% of humic acid had significantly better economical efficiency ($P \leq 0.05$) followed by those fed basal diet supplemented with 0.2% and 0.4 % than the control group.

Results of present study are in accordance with the reports of (Shermer *et al.*, 1998; Ozturk *et al.*, 2010; Nagaraju *et al.*, 2014 and Ozturk *et al.*, 2014) who found that use of humic acid on daily basis showed positive effect on broilers growth performance. In another experiment, Arif *et al.* (2016) indicated that humic acid in diet improved starter and finisher weight gain body weight and feed efficiency. Similarly, Avci *et al.* (2007) and Salah *et al.* (2015) reported that humates supplemented to broiler diets improved body weight gain, feed conversion ratio. Perhaps, humic acid leads to stabilize animal gut micro flora and result in improved nutrient absorption and weight gain (Shermer *et al.*, 1998). Positive effects on growth of broiler which chickens were found by using humic acids in diet were obtained by other researcher (Pistova *et al.*, 2016). Furthermore, Arafat *et al.* (2015) postulated that the supplementation of humic acid in drinking water improved FCR of laying hens. This finding is also in agreement with the improvement of FCR found in other studies in which humic substances were supplemented either in the drinking water of broiler chickens (Ozturk *et al.*, 2010) or in the diet (Rath *et al.*, 2006 and Taklimi *et al.*, 2012).

Some mode of action have been proposed to clarify the advantages found in poultry diet supplemented with humic substances. From the studies of Taklimi *et al.* (2012), it is suggested that the advantages of humic substances are expected to be no less than four mode of action: i) The capacity to make defensive layers over the epithelial mucosal film of the gut against the passage of toxic and other bacterial contaminated substances; ii) The capacity to reduce the pH of the digestive tract may have led to the repression of intestinal microscopic organisms leading to the decrease metabolic needs and increase metabolism of protein and microbial carbohydrates, thereby increasing the availability of nutrients; iii) The ability to reduce the absorption of nitrates, fluorites and heavy metals, thereby detoxification in the gut and iv) Increasing immune receptors in the gut lining to protect against pathogens, promote growth. Furthermore, the supplementation of humic substances increased relative lymphocyte counts in

pigs (Wang *et al.*, 2008). The authors demonstrated that humic substances may show a valuable impact on animal immune system, in association with their capability to form complex saccharides in the body, which function as modulators of intercellular interaction. This improvement in FCR may be caused by effects of the decrease in total bacterial count, *Salmonella*, *E.Coli* and *Proteus* by using humic acid.

On the other hand, the improvement in the FCR with humic acid supplementation could be possibly due to better utilization of nutrients resulting in increased body weight (Lala *et al.*, 2016). Organic acids improve the absorption and conversion of nutrients in the body, and improve overall gastric function (Park *et al.*, 2009).

Table 2. Performance of broiler Sasso fed diet supplemented with humic acid

Items	Control	Humic acid			Sig	SEM
		0.1 %	0.2 %	0.4 %		
Live body weight (g) at:						
7 d	128	128	127	127	0.924	4.47
28 d	689 ^d	948 ^a	911 ^b	853 ^c	0.005	25.15
51 d	1711 ^d	2118 ^a	1981 ^b	1948 ^c	0.003	29.99
Body weight gain (g) from:						
7-28d	562 ^d	820 ^a	784 ^b	726 ^c	0.001	24.44
29-51d	1021 ^c	1169 ^a	1071 ^b	1094 ^b	0.001	30.08
7-51d	1583 ^d	1990 ^a	1855 ^b	1821 ^c	0.001	29.39
Feed intake (g) from:						
7-28d	1375 ^a	1179 ^c	1195 ^c	1217 ^b	0.01	16.41
29-51d	2755 ^a	2213 ^d	2309 ^c	2383 ^b	0.009	18.88
7-51d	4130 ^a	3392 ^d	3505 ^c	3600 ^b	0.009	22.65
Feed conversion ratio (g feed/g gain) from age:						
7-28d	2.46 ^a	1.45 ^d	1.53 ^c	1.69 ^b	0.002	0.06
29-51d	2.72 ^a	1.90 ^c	2.17 ^b	2.19 ^b	0.005	0.07
7-51d	2.62 ^a	1.71 ^d	1.89 ^c	1.98 ^b	0.003	0.04
Economical efficiency and production index:						
Economic efficiency	21.5 ^c	70.3 ^a	50.7 ^b	48.8 ^b	0.001	1.77
Production index	129 ^d	243 ^a	206 ^b	193 ^c	0.009	4.13

^{a,b,c,d} Means in the same row followed by different letters are significantly different at ($p \leq 0.05$); SEM, Standard error of mean.

The apparent digestibility of the nutrients

The apparent digestibility of the nutrients of broiler chickens fed diet supplemented with humic acid during days 7-51 of age are shown in Table 3. Chicks fed basal diet supplemented with humic acid at different levels had significantly ($p \leq 0.05$) better digestibility values of crude protein and ether extract than the control group without significant differences among the different levels of humic acid. While, those fed basal diet supplemented with 0.1% of humic acid had significantly higher digestibility of crude fiber than control group and 0.2% of humic acid group. Furthermore, chicks fed basal diet supplemented with 0.1 and 0.2% of humic acid had

significantly higher digestibility of dry matter than only the control group. However, there were no significant differences in apparent ash retention% among supplements and control group. The improvements in the apparent digestibility of the nutrients with humic acid in diet were obtained by (Sheikh *et al.*, 2010). Results of present study are in the same line with those of Pisarikova *et al.* (2010) who reported that humic substances supplementation led to improved the ileal digestibility of crude protein and crude fat and had an inclination for increased ileal digestibility of dry matter, crude fiber and ash in growing pigs.

Table 3. The apparent digestibility of the nutrients of broiler Sasso fed diet supplemented with humic acid

Items	Control	Humic acid			Sig	SEM
		0.1 %	0.2 %	0.4 %		
Crude protein	69.03 ^b	80.64 ^a	78.32 ^a	78.05 ^a	0.017	2.40
Ether extract	70.20 ^b	84.30 ^a	83.31 ^a	82.69 ^a	0.003	2.53
Crude fiber	17.19 ^b	20.85 ^a	16.62 ^b	19.57 ^{ab}	0.048	1.10
Apparent Ash retention, %	31.24	36.85	35.09	35.89	0.275	2.07
Dry matter	68.96 ^b	74.99 ^a	73.91 ^a	72.55 ^{ab}	0.022	1.28

^{a,b} Means in the same row followed by different letters are significantly different at ($p \leq 0.05$); SEM, Standard error of mean.

When humic substances supplemented to broiler diet leads to increasing the length of both mucosal villi of the jejunum and gut length (Taklimi *et al.*, 2012) has been related with improved digestibility of nutrients due to a diminishment of the passage rate of the intestinal content and increasing extension of enzymatic digestion. Besides, in rats supplemented with humic substances, the advantages in weight gain and nitrogen retention found in two investigations were related with a greater area of the epithelial surface, higher length of the villi and greater crypt depth (Yasar *et al.*, 2002).

This may be explained on the basis that humic acid stabilises the intestinal microflora and thus ensures increased nutrient absorption and an improved utilisation of nutrients in animal feed (Shermer *et al.*, 1998).

The blood constituents:

The biochemical blood constituents of broilers fed diets supplemented with humic acid are shown in Table 4. Chicks fed basal diet supplemented with 0.1% of humic acid had significantly higher glucose than other treatments without differences were recorded among 0.1 and 0.2% humic acid. Supplementation of humic acid decreased serum AST, ALT, urea and creatinine and increased urea /creatinine ratio compared to control group. In addition, all levels of humic acid decreased serum total lipids, triglycerides, cholesterol, HDL and LDL compared to control group. Moreover, Chicks fed basal diet supplemented with 0.1% humic acid had significantly lower ALT and ALT/AST ratio and higher creatinine and HDL than other supplements. No significant differences were recorded in urea, AST, triglycerides, cholesterol and LDL among the different levels of humic acid. Moreover, Chicks fed basal diet supplemented with humic acid at different levels had significantly higher T4 than the control group. On the other hand, antioxidants enzymes including GPX and SOD were higher in chickens fed basal diet supplemented with humic acid at different levels compared to the control group. However, there were no significant differences in Alkaline phosphatase, T3, TAC and GSH among supplements and control group. These results approach with those reported by Abdel-Mageed (2012) and Salah *et al.* (2015) who found that broiler chickens supplemented with humic acid showed significant decreases in AST and ALT beside insignificant decrease of uric acid and creatinine. Also, Rath *et al.* (2006) found that blood urea nitrogen was significantly reduced by humic substances supplementation. A similar conclusion was drawn by Hanafy and El-Sheikh, (2008) who showed that humic acid supplementation had no significant effect on plasma T3 concentrations. Moreover, Arif *et al.* (2016) reported that LDL, the harmful cholesterol, was significantly decreased in humic acid supplemented diets. Lessening in blood cholesterol and lipids may be because of reduction in microbial intracellular pH (Abdo, Zeinb, 2004). By discouragement of microbial enzymes, bacterial

cell film is forced to use energy to release acidic protons which causing reduce in intracellular pH (Young and Foegeding, 1993). Contradictive results were obtained by Rath *et al.* (2006) who found that blood glucose was significantly reduced by humic substances supplementation. Also, Avci *et al.* (2007) reported that blood glucose, triglycerides and LDL were not affected by humic acid and humic substances in broiler and quails diets. Similarly Arif *et al.* (2016) found that humic acid supplementation did not affect ($P > 0.05$) blood glucose, urea and creatinine. A similar conclusion was drawn by Rensburg *et al.* (2006) and Hanafy and El-Sheikh (2008) who showed that humic acid supplementation had no significant effect on plasma Albumin, AST and ALT concentrations. This disagreement may be caused by effects of several factors such as humic acid sources, birds species, rearing of animal in various regions of the world differing in the climate.

Feeding diet with different levels of humic acid increased RBC's hemoglobin and WBC's and decreased MCV and MCH compared to control group. No significant differences were recorded in RBC's hemoglobin, WBC's, MCV and MCH among the different levels of humic acid. Moreover, there were no significant differences in PCV, MCHC, Lymphocytes, Monocytes, Basophils, Eosinophils and Heterophiles among supplements and control group (Table 5). Our results herein are consistent with the findings of Hanafy and El-Sheikh (2008) who found that RBC's, WBC's and hemoglobin were significantly ($P \leq 0.05$) increased for hens fed humic acid supplementation compared with the control group. Also, Ipek *et al.* (2008) showed that humic acid addition increased RBC and hemoglobin in Japanese quail. According to Cetin *et al.* (2006) addition of humic substances to laying hens diet had no effects on PCV, but affected, RBC and hemoglobin. Similarly, Banaszkiwicz and Drobnik (1994) found that increase of globulin, hemoglobin and RBC were found in rats treated with humic substances. This increase was probably due to that humic substances, when binding inorganic ions, facilitates the transport of these minerals (Islam *et al.*, 2005). Humic substances may therefore enhance the ability of the body to utilize nutrients by participating in composition of RBC and hemoglobin.

Results are also contradictory to the findings of Rath *et al.* (2006) and Ipek *et al.* (2008) suggested that humic acid did not have any effect on WBC's in broiler chickens or Japanese quail, respectively. Furthermore, Miceta *et al.* (2012) postulated that humic substances supplementation to diets of growing rabbits did not change the erythrocytic indices. Also, Arif *et al.* (2016) reported that no significant influence ($P > 0.05$) was suggested in RBC's, WBC's and hemoglobin. It might be attributed to different age and strains of broiler (Talebi *et al.*, 2005).

Table 4. Biochemical parameters of blood serum of broiler sasso fed diet supplemented with humic acid

Items	Control	Humic acid			Sig	SEM
		0.1 %	0.2 %	0.4 %		
Glucose (mg/dl)	179.60 ^b	185.20 ^a	183.20a ^b	180.20 ^b	0.021	2.263
Urea (mg/dl)	25.50 ^a	20.80 ^b	20.00 ^b	21.20 ^b	0.001	0.397
Creatinine (mg/dl)	1.220 ^a	0.840 ^b	0.740 ^c	0.680 ^c	0.007	0.032
Urea/ Creatinine	20.95 ^c	24.83 ^b	27.07 ^b	31.54 ^a	0.001	0.996
ALT(U/L)	65.00 ^a	57.40 ^c	62.60 ^b	61.00 ^b	0.001	0.869
AST(U/L)	60.48 ^a	55.70 ^b	54.60 ^b	55.20 ^b	0.001	0.886
ALT/AST	1.08 ^c	1.03 ^d	1.15 ^a	1.11 ^b	0.001	0.009
Alkaline phosphatase (U/100ml)	12.60	11.80	12.80	11.80	0.169	0.381
Total Lipid (mg/dl)	47.80 ^a	39.00 ^c	41.60 ^b	40.80 ^{bc}	0.002	0.731
Triglycerides (mg/dl)	188a	175 ^b	176 ^b	176 ^b	0.006	2.580
Cholesterol (mg/dl)	217 ^a	207 ^b	205 ^b	206 ^b	0.045	2.981
HDL(mg/dl)	41.80 ^a	39.00 ^b	35.20 ^c	37.00 ^c	0.001	0.628
LDL(mg/dl)	98.40 ^a	89.40 ^b	90.00 ^b	90.00 ^b	0.005	1.334
TAC (mg/dl)	410	424	414	419	0.380	5.954
GPX (mg/dl)	40.20 ^c	47.00 ^a	47.00 ^a	43.80 ^b	0.001	0.738
GSH (mg/dl)	970	973	960	963	0.889	13.680
SOD (mg/dl)	242 ^c	264 ^a	254 ^{ab}	251 ^{bc}	0.005	3.669
T3 (ng / ml)	219	223	222	221	0.861	3.180
T4 (ng / ml)	13.20 ^b	16.80 ^a	17.60 ^a	18.20 ^a	0.005	0.566

^{a,b,c,d} Means in the same row followed by different letters are significantly different at ($p \leq 0.05$); SEM=Standard error of mean; AST=aspartate amino transferase; ALT=alanine amino transferase; HDL=high-density lipoprotein; LDL=low-density lipoprotein; T3= triiodothyronine; T4=thyroxine; TAC=total antioxidant capacity; GPX =glutathione peroxidase; GSH= glutathione; SOD=superoxide dismutase.

Table 5. Hematological traits of broiler sasso fed diet supplemented with humic acid.

Items	Control	Humic acid			Sig	SEM
		0.1 %	0.2 %	0.4 %		
RBC's ($10^6/\text{cmm}^3$)	12.80 ^b	15.40 ^a	15.20 ^a	15.80 ^a	0.002	0.265
Hemoglobin (g/100ml)	10.80 ^b	11.80 ^a	12.20 ^a	11.80 ^a	0.027	0.300
PCV %	32.80	33.00	34.40	33.00	0.421	0.742
MCV	254 ^a	216 ^b	224 ^b	210 ^b	0.003	4.69
MCH (Ug)	84.2 ^a	76.8 ^b	81.0 ^{ab}	75.0 ^b	0.015	1.91
MCHC (%)	32.80	35.80	36.00	35.40	0.149	1.042
WBC's ($10^3/\text{cmm}^3$)	23.00 ^b	26.20 ^a	25.00 ^a	26.00 ^a	0.002	0.510
Lymphocytes (%)	40.40	43.40	42.60	43.80	0.093	0.951
Monocytes (%)	15.40	15.40	15.20	13.80	0.206	0.592
Basophils, (%)	0.800	0.400	0.800	0.800	0.468	0.212
Eosinophils, (%)	10.80	9.80	9.80	9.40	0.182	0.442
Heterophiles, (%)	32.60	31.00	31.60	32.20	0.788	1.179

^{a,b,c,d} Means in the same row followed by different letters are significantly different at ($p \leq 0.05$); SEM= Standard error of mean; RBC's=red blood cell; PCV=packed cell volume; MCH=mean corpuscular hemoglobin; WBC's=white blood cell, MCV=Mean cell volume, MCHC= Mean Corpuscular Hemoglobin Concentration

Feeding diet with different levels of humic acid increased total protein, globulin- γ , LA, BA, LTT, phagocytic activity and phagocytic index and decreased albumin/globulin ratio, α -globulin and β -globulin compared to control. While, chicks fed basal diet supplement with 0.1 of humic acid had significantly higher globulin- γ , phagocytic activity and phagocytic index and lower β -globulin than other levels supplements and control group. On the other hand, Chicks fed basal diet supplemented with 0.2 of humic acid had significantly higher albumin than other levels of humic acid (Table 6). Moreover, there were no significant differences in IgA, IgM and IgG among supplements and control group. Supported to our results, Hanafy and El-Sheikh (2008) indicated that plasma total protein concentrations significantly ($P \leq 0.05$) increased for hens fed high level of humic acid compared to

other groups. Salah *et al.* (2015) found that broiler chickens supplemented with humic acid showed significant increases in leukocytic count, lymphocyte, phagocytosis, phagocytic index and total proteins. Ertaş *et al.* (2006) reported that humic acid improved protein digestion in Japanese quail. Also, Cetin *et al.* (2011) suggested that addition of humic acid (0.15%) in laying hens diet resulted in significant increases in the lymphocyte counts via the increased production of IL-2 and the expression of IL-2 receptors on lymphocyte which resulted in the enhancement of the activity of IL-2 producing cells. In this connection, Terratol (2002) also suggested that humic acid may stimulate the production of glycoproteins, which can regulate the immune system via the maintenance of the balance of killer and T cells.

Table 6. Immune indices of broiler sasso fed diet supplemented with humic acid

Items	Control	Humic acid			Sig	SEM
		0.1 %	0.2 %	0.4 %		
Total protein (g/dl)	5.90 ^b	6.42 ^a	6.42 ^a	6.34 ^a	0.005	0.100
Albumin (g/dl)	3.18 ^a	2.96 ^b	3.24 ^a	2.90 ^b	0.001	0.056
Globulin (g/dl)	2.72 ^c	3.46 ^a	3.18 ^b	3.44 ^a	0.004	0.059
A/G ratio	1.174 ^a	0.856 ^c	1.018 ^b	0.842 ^c	0.002	0.022
α -globulin (g/dl)	1.30 ^a	1.08 ^b	1.08 ^b	1.08 ^b	0.006	0.028
β - globulin (g/dl)	1.00 ^a	0.544 ^d	0.636 ^c	0.738 ^b	0.001	0.023
γ -Globulin (g/dl)	0.42 ^d	1.84 ^a	1.46 ^c	1.62 ^b	0.005	0.043
LA (IU %)	9.00 ^b	11.40 ^a	12.20 ^a	11.80 ^a	0.003	0.430
BA (%)	34.80 ^b	41.00 ^a	40.20 ^a	40.40 ^a	0.001	0.640
LTT(%)	21.40 ^b	24.80 ^a	25.00 ^a	25.00 ^a	0.002	0.500
PI (%)	15.00 ^d	21.20 ^a	17.20 ^c	19.20 ^b	0.007	0.574
PA (%)	15.00 ^c	21.20 ^a	18.20 ^b	19.20 ^b	0.002	0.424
IgA (mg/100 ml)	78.60	79.60	80.80	78.60	0.523	1.185
IgG (mg/100 ml)	970	969	954	961	0.829	13.611
IgM (mg/100 ml)	226	232	228	231	0.576	3.284

^{a,b,c,d} Means in the same row followed by different letters are significantly different at ($p \leq 0.05$); SEM= Standard error of mean; PA= Phagocytic activity; PI= Phagocytic index; LA= lysozyme activity; BA= Bactericidal activity; LTT= Lymphocyte transformation test; IgA= Immunoglobulin A; IgG= Immunoglobulin G; IgM= Immunoglobulin M

Carcass characteristics :

Feeding diet with 0.1 and 0.2% of humic acid increased significantly percentage of dressing and total edible parts and decreased percentage of inedible parts compared to control (Table 7). While, Feeding diet with 0.1 % of humic acid increased significantly percentage of heart and proventriculus compared to other levels of humic acid and control. Furthermore, feeding diet with 0.2% of humic acid increased percentage of gizzard compared to control. On the other hand. Feeding diet with 0.4 % of humic

acid increased significantly percentage of intestinal weight compared to other levels of humic acid and control. However, feeding diet with different levels of humic acid decreased percentage of abdominal fat, intestinal length and pancreas compared to control (Table 7). Similar to the findings of the present study, Mirnawati and Marida (2013) and Abdel-Mageed (2012) noted that birds given diets with humic substances had significant increase in dressing, breast and thighs % and significant decrease in abdominal fat % as compared to those fed control diet.

Table 7. Carcass characteristics , relative weight of immune organs and chemical composition of meat of broiler sasso fed diet supplemented with humic acid.

Items	Control	Humic acid			Sig	SEM
		0.1 %	0.2 %	0.4 %		
Carcass characteristics :						
Dressing, %	73.30 ^b	82.06 ^a	82.20 ^a	77.78 ^{ab}	0.017	1.97
Total edible parts, %	77.42 ^b	85.86 ^a	85.90 ^a	81.02 ^{ab}	0.027	2.061
Inedible parts, %	22.58 ^a	14.14 ^b	14.10 ^b	18.98 ^{ab}	0.027	2.061
Heart, %	0.471 ^b	0.598 ^a	0.339 ^c	0.449 ^b	0.004	0.013
Gizzard, %	1.279 ^b	1.199 ^b	1.638 ^a	0.953 ^c	0.003	0.034
Proventriculus, %	0.316 ^c	0.520 ^a	0.443 ^b	0.331 ^c	0.004	0.01
Liver, %	2.32 ^a	2.02 ^{ab}	1.75 ^b	1.86 ^b	0.01	0.109
Abdominal fat, %	1.86 ^a	0.884 ^b	0.677 ^b	0.602 ^b	0.005	0.18
Intestinal Weight, %	4.12 ^b	2.97 ^d	3.67 ^c	5.07 ^a	0.001	0.141
Intestinal length, %	2.10 ^a	1.77 ^b	1.92 ^b	1.86 ^b	0.007	0.058
Pancreas, %	0.202 ^a	0.170 ^b	0.175 ^b	0.145 ^c	0.009	0.004
Immune organs :						
Spleen, %	0.219 ^b	0.322 ^a	0.141 ^c	0.232 ^b	0.001	0.005
The bursa of Fabricius, %	0.065 ^c	0.101 ^b	0.141 ^a	0.065 ^c	0.001	0.003
Thymus, %	0.275 ^c	0.163 ^d	0.435 ^b	0.652 ^a	0.008	0.011
Chemical composition of meat :						
Protein , %	23.00 ^b	26.00 ^a	24.00 ^{ab}	24.00 ^{ab}	0.031	0.645
Fat, %	2.900 ^a	2.100 ^c	2.400 ^b	2.30 ^b	0.002	0.066
Ash, %	12.80 ^a	12.10 ^{ab}	11.50 ^b	11.60 ^b	0.042	0.32
TAC (mg/dl)	426	440	436	436	0.849	11.573

^{a,b,c,d} Means in the same row followed by different letters are significantly different at ($p \leq 0.05$); SEM=Standard error of mean, TAC =Total antioxidant capacity

Immune organs :

Feeding diet with 0.1 % of humic acid increased significantly percentage of spleen compared to other levels of humic acid and control. Also, feeding diet with 0.2% of humic acid increased percentage of bursa compared to control. On the other hand, feeding diet with 0.4 % of humic acid increased significantly percentage of thymus compared to other levels of humic acid and control. Our results herein are consistent with the findings of Rath *et al.* (2006) who, suggested that the relative weights of the bursa of fabricius increased in birds given 0.25% humate suggesting a possible immunostimulatory impact that has been considered to be an impact of humate. Humic acid may exert a beneficial impact on immune systems of birds. Also, Hanafy and El-Sheikh (2008) proposed that relative weight of spleen significantly ($P \leq 0.05$) increased for hens fed high level of humic acid (200 mg) compared with the control group. The results obtained from this study show that the increase of relative weight of spleen and white blood cells as result of humic acid addition could play a role in improving the immune function. These results approach with those proposed by Rath *et al.* (2006) who suggested that the relative weights of bursa of fabricius increased in chickens given 2.5 % humic acid suggesting an immunostimulatory impact that has been proposed to be an effect of humic acid. Moreover, Joone *et al.* (2003) proposed that humic acid having immunostimulatory, anti-inflammatory and antiviral effects. Results obtained here in are also contradictory to Avci *et al.* (2007) who suggested that no significant differences in slaughter characteristics were found between birds fed diet

with humate or humic acid compared with the control group in broiler chickens or Japanese quails.

Chemical composition of meat:

Feeding diet with 0.1 % of humic acid increased protein in meat compared to control group. However, Chicks fed basal diet supplemented with 0.1 % of humic acid had significantly lower fat than other supplements and control group. This improvement in the meat quality and result in healthy meat for human consumption. While, no significant differences between groups in total antioxidant capacity (TAC) (Table 7).

All levels of humic acid decreased total bacterial count, *Salmonella*, *E.Coli* and *Proteus* compared to control group. However, Chicks fed basal diet supplemented with 0.1 of humic acid had significantly lower count of *Salmonella*, *E.Coli* and *Proteus* than the other supplemented groups (Table 8). Similar to the results of the present study, Abdel-Mageed (2012) proposed that feeding diets with humic substances resulted in significant reduce in coliform, *Escherichia coli* and *Clostridium perfringes* of the intestinal content as well as intestinal pH as compared to control diet. The humic substances can form a protective membrane on the mucus epithelium of the gut tract against infections and toxins, thus ensuring an improvement in utilization of nutrients in animal feed (Islam *et al.*, 2005). Huck *et al.* (1991) suggested that humic substances may influence, in particular, the metabolism of proteins and carbohydrates in microbes. This results are in a direct devastation of bacterial cells or viral particles, which should result in improved growth performance.

Table 8. Bacterial count of broiler sasso fed diet supplemented with humic acid

Items	Control	Humic acid			Sig	SEM
		0.1 %	0.2 %	0.4 %		
TBC (cfu)	2.675 ^a	2.024 ^b	2.113 ^b	2.127 ^b	0.001	0.055
<i>Salmonella</i> (cfu)	0.925 ^a	0.805 ^c	0.845 ^b	0.841 ^b	0.003	0.010
<i>E.Coli</i> (cfu)	1.135 ^a	0.849 ^b	0.875 ^b	0.841 ^b	0.001	0.032
<i>Proteus</i> . (cfu)	0.870 ^a	0.543 ^b	0.354 ^c	0.396 ^c	0.006	0.030

^{a,b,c,d} Means in the same row followed by different letters are significantly different at ($p \leq 0.05$); SEM, Standard error of mean, TBC = Total Bacterial Count

CONCLUSION

Humic acid supplementation at 0.1, 0.2 and 0.4% improved productive performance, some blood parameters, carcass characteristic and immune response especially at the level of 0.1%, without any adverse effects on blood components of Sasso chicken.

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تأثير اضافة حمض الهيوميك في العليقة على أداء النمو وبعض مكونات الدم وخصائص الذبيحة في دجاج الساسو أسماء شوقي النجار¹، محمود إبراهيم الكيلوي²

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أجريت هذه الدراسة لتقييم تأثير حمض الهيوميك على أداء النمو وخصائص الذبيحة والكفاءة الاقتصادية لدجاج الساسو. عند عمر أسبوع تم توزيع عدد مائة ستة وتسعون طائر ساسو إلى أربع مجموعات (٤٩ طائر بالمجموعة في سبع مكررات). غذيت الكتاكيت على العليقة الأساسية مع تقديم المعاملات الغذائية التالية: المجموعة الأولى غذيت على العليقة الأساسية بدون اي اضافة (مجموعة مقارنة)، أما المجموعات الثانية والثالثة والرابعة فقد تغذت على العليقة الأساسية مع إضافة مع ٠.١ و ٠.٢ و ٠.٤٪ من حمض الهيوميك. في نهاية التجربة، تم قياس بعض خصائص الذبيحة وتم أخذ عينات الدم لتحديد بعض مكونات بلازما الدم. وأظهرت النتائج أن دجاج الساسو المغذاه على ٠.١٪ من حمض الهيوميك أظهر أفضل أداء إنتاجي و كفاءة اقتصادية مقارنة بالمجموعة الكنترول. الكتاكيت المغذاه على ٠.١٪ من حمض الهيوميك كانت معنويًا أعلى في الجلوكوز من مجموعة السيطرة. أدت إضافة حمض الهيوميك إلى خفض نشاط انزيم ناقل الألانين (ALT) ونشاط انزيم ناقل الاسبارتات (AST) و اليوريا و الكرياتينين و الدهون الكلية والدهون الثلاثية، الكولسترول و الكولسترول عالي ومنخفض الكثافة ومتوسطكمية هيوجلوبينكريتية الدم الحمراء (MCH) حجم الكرية الحمراء (MCV) والألفا والبيتا الجلوبيولين، وزيادة الثيروكسين و نشاط انزيمات الأكدسة GPX و SOD و عدد كرات الدم الحمراء و عدد كرات الدم البيضاء والهيموجلوبين والبروتين الكلي والجاما الجلوبيولين و معامل تحويل الخلايا الليمفاوية (LTT) ونشاط مقاومة البكتريا و نشاط الليزوزيم والنشاط البلعبي ودليل النشاط البلعبي مقارنة بمجموعة الكنترول (في المدى الطبيعي). أدت التغذية على ٠.١ و ٠.٢٪ من حمض الهيوميك إلى زيادة معنوية في نسبة التصافي وانخفاض الدهون في منطقة البطن مقارنة مع الكنترول. وعلاوة على ذلك، أدت إضافة حمض الهيوميك إلى خفض العد البكتيرية بالجهاز الهضمي مقارنة مع مجموعة الكنترول.

إضافة حمض الهيوميك بمعدل ٠.١ و ٠.٢ و ٠.٤٪ أدت إلى تحسن أداء النمو، ومعاملات هضم العناصر الغذائية، ودليل الإنتاج والكفاءة الاقتصادية وخاصة عند مستوى ٠.١٪، دون أي آثار سلبية على مكونات الدم في دجاج الساسو.