



PRODUCTIVE AND PHYSIOLOGICAL RESPONSE OF BROILER CHICKS TO DIETARY HUMIC ACID

A. A-Ghazalah¹; El-Tahawy W. S.²; Asmaa A., Ghalwash² and
Asmaa Sh. Elnaggar²

1- Dept. of Anim. Prod., Fac. of Agric, Cairo Uni.

2- Dept. of Anim. and Poult. Prod., Fac. of Agric., Damanhour Uni.

Corresponding author: Asmaa Sh. Elnaggar; Email: asmaa.elnaggar@agr.dmu.edu.eg

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ABSTRACT: An experiment was carried out to investigate the effect of dietary humic acid supplementation on performance of broiler chicks. One hundred and eighty Arbor Acres broiler chicks were assigned equally into five treatment groups. Humic acid was supplemented to basal diet 0 (control), 1.00, 2.00, 3.00 and 4.00 g/kg respectively. Chicks fed diet with different levels of humic acid had significantly ($P < 0.05$) greater production performance than the control group. Humic acid had significantly improved the digestibility of crude protein and ether extract. Feeding diet with humic acid significantly decreased plasma content of cholesterol, malondialdehyde (MDA) and low-density lipoprotein (LDL) while increased glucose, total protein, triiodothyronine, thyroxine, glutathione, globulin, γ globulin, compared to control group. Humic acid significantly increased the percentage of dressing and decreased abdominal fat compared to control. In conclusion, humic acid supplementation at 1.00, 2.00 and 3.00 and 4.00 g/kg diet was superior regarding growth, digestibility, and economical return, without negative effects on blood and carcass traits of broilers.

Keywords: Broiler, Humic acid, Growth, Digestibility, Blood .

INTRODUCTION

It is well known that including antibiotics in poultry diets results in health problems leading to bacteria resistance against medications administered by humans (Arif *et al.*, 2016). In poultry new supplements such as probiotics, prebiotics, humates and enzymes have been suggested in order to increase the efficiency of feed utilization and maintain the general health status of birds (Levy, 2014). Humic acid (HA), have been recommended to raise the profitability and provided consumers with healthy products of poultry (Griggs and Jacop, 2005).

previous studies showed the capacity of HA to inhibit bacterial growth, improve the immune system, antiviral properties, as well as to prevent and care intestinal disorders, improve the nutritive value of feed and trace element utilization, with positive effects on growth performance and a reduction in mortality (Nagaraju *et al.*, 2014 and Mudroňova *et al.*, 2020).

Several studies reported that humate is not toxic and contains no carcinogenic substance (Yasar *et al.*, 2002). Besides, humic acid contain some trace elements which act as co-factors for several enzymes which increase digestion and utilization of nutrients (Hayirli *et al.*, 2005).

The addition of humic acid to poultry diets improves the immune status, plays a role in uplifting liver functions (Islam *et al.*, 2005) and stimulates the production of lymphocytes (Joone, and Van Rensburg, 2003). Humic acid has a promoting effect on digestive system and productive performance of broilers. Oztürk *et al.* (2012) obtained an increase in live weight and a decrease in blood cholesterol levels due to supplementing humic acid to broiler diet. The use of humic acid maintains the health of poultry digestive system, therefore, enhances their productive performance (Windisch *et al.*, 2008).

Humic acid has a powerful role in maintaining the health of the gastrointestinal tract of poultry, thus improving their performances (Rath *et al.*, 2006 and Windisch *et al.*, 2008). Broiler chickens supplemented with humic acid showed improved body weight gain, feed conversion ratio as well as economical efficiency. Moreover, HA increases leukocytic count, phagocytosis, phagocytic index, total proteins γ globulin, (Salah *et al.*, 2015, ELnaggar and El-Kelawy (2018). Perhaps, humic acid leads to stabilize animal gut micro flora and result in improved nutrient absorption and weight gain (Pistova *et al.*, 2016).

Therefore, this study aimed to evaluate the impact of dietary supplementation of humic acid on productive and physiological responses of broiler chicks.

MATERIALS AND METHODS

The current experiment was conducted at the Animal and Poultry Research Centre (El-Bostan Farm), which is part of the Animal and Poultry Production Department, Faculty of Agriculture, Damanhour University. All treatments and birds care procedures were approved by the Institutional Animal Care and Use Committee in Damanhour University, Egypt

Birds and experimental design

A total number of 180 unsexed Arbor Acres broiler chickens at 7 days of age, obtained from a commercial hatchery were randomly distributed into five groups, each of six replicates, six birds per replicate. They were reared in similar hygienic and managerial conditions. After the first week, chicks were submitted to five dietary treatment groups. The first group was fed the basal diet without supplementation (control); while the 2nd, 3rd, 4th and 5th groups were fed basal diet supplemented with different levels of humic acid being 1.00 , 2.00, 3.00, and 4.00 g/ kg diet respectively. The experimental diets were

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formulated according to the strain management guide as shown in Table (1).

Housing and husbandry

Chicks were housed in breeding pens in semi-opened house. They were fed, *ad libitum*, the experimental diets and given free access to water. A light schedule similar to commercial condition included 23 h light until the 7th day followed by 20 h light from the 8th day until the end of the experimental period was provided. Average outdoor minimum and maximum temperature and relative humidity during the experimental period was 20C° and 27 C° and 55.7 % and 58.7%, respectively. The brooding temperature (indoor) was declined gradually, being 32, 30, 27 and 21-24 C° during 1-7, 8-14, 15-20 and 21-39 days of age, respectively.

Data collection

Performance parameters including individual live body weight (LBW, g), and feed consumption (FC, g) were recorded throughout the trial period (7-39 d of age). For each replicate within treatment groups, accordingly, body weight gain (BWG, g) and feed conversion ratio (feed/gain ratio, FCR) were calculated. Apparent digestibility of dry matter, crude protein, ether extract and crude fiber were done using five birds per treatment housed individually in metabolic cages using total collection method as cited by Abou-Raya and Galal (1971). Nitrogen, ether extract, crude fiber and dry matter of the excreta as well as those of feed were determined according to AOAC (2004). The economic efficiency of experimental diets was estimated as the ratio between income and total feed cost during the experimental growth period (Zeweil, 1996). The price of the diets and humic acid supplements was calculated according to the local market price at the same time as the experiment in 2021 by the Egyptian pound (L.E.).

Economic efficiency = (Net revenue/ Total feed cost) *

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

Net revenue = Total revenue - Total feed cost.

Total revenue: the selling price of the obtained live weight

European Production Efficiency Index (EPEI) according to **Hubbard broiler management guide 1999**

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

Where:

BW = Body weight (kg), SR = Survival rate (100% - Mortality), PP = Production period (days), FCR = Feed conversion ratio (kg feed / kg gain). Before slaughter, 3 mL of blood was drawn from the wing vein and placed in un-heparinized vacuum tubes. Coagulated blood samples were centrifuged for 15 minutes at 4000 rpm, and the clear serum was extracted and stored at -20°C until biochemical analysis. According to Fossati and Prencipe's (1982), serum total lipids and serum triglycerides concentrations were tested using specific kits from CAL-TECH Diagnostics, INC, (CAL) Chino, California, U.S.A. According to the recommendations of Bogin and Keller (1987), serum total cholesterol was estimated using the specific kits, high-density lipoprotein (HDL) was measured according to the method outlined by Lopez-Virella (1977), and low-density lipoprotein (LDL) was calculated by the formula of Friedewald *et al.* (1972). Serum glucose concentration was measured by the method of Trinder (1969). total protein (g/dl) according to Henry *et al.* (1974), albumin (g/dl) according to Doumas (1971), and different types of globulin (α , β and γ -globulin) according to Bossuyt *et al.*(2003), besides, serum globulin concentration was calculated by difference. Serum concentration of total tri-iodothyronine (T₃) and thyroxin (T₄)

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was assayed by radioimmunoassay technique using the kit from Diagnostic Products Corporation, Los Angeles, USA. The activity of malondialdehyde (MDA) in the blood was measured using the method reported by Placer *et al.* (1966). The colorimetric method of Koracevic *et al.* (2001) was used to assess blood total antioxidant capacity (TAC). Serum glutathione peroxidase activity was determined according to the colorimetric method of Bauer (1982). Misra and Fridovich (1972) method for measuring serum superoxide dismutase (SOD) was used.

Six chicks from each treatment were chosen at random at the end of the experiment (39 days) and slaughtered after a 12-hour fastening period to determine carcass characteristics. Abdominal fat was removed from the gizzard and abdominal region, and each carcass was individually weighed and estimated relative to the pre-slaughtered weight. Individual lymphoid organs (spleen, thymus, and bursa) were removed, weighed, and the weight of each organ was estimated relative to the pre-slaughtered weight.

Statistical analysis
Data were subjected to the one-way ANOVA procedure using statistical analysis system (SAS, 2006) with the following model:

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

Where Y_{ij} = is the dependent variable; μ = the general mean; T_i = the fixed effect of treatment and e_{ij} = experimental random error. The difference among means was determined using Duncan's new multiple range test (Duncan, 1955) at $P < 0.05$.

RESULTS AND DISCUSSION

Productive performance traits

Productive traits including live body weight (LBW), body weight gain (BWG), feed consumption (FC), feed conversion ratio (FCR) of broiler chicks during 7d to 39d are involved in Table (2).

There were significant differences among treatments at 39nd days of age compared to the control group. At 39 days of age, body weight of chickens fed diet supplemented with different levels of HA were significantly heavier compared to the control with no significance among levels of humic acid. Similarly, during 7-39 days of age, chickens fed diet supplemented with different levels of HA had higher ($P < 0.05$) body weight gain compared to the control with no significance between different levels of HA. There were no significant differences in FC by adding different levels of HA to chick diets during the study period. The results indicated that addition of different levels of humic acid as a natural growth promoter improved significantly ($P < 0.05$) in feed conversion ratio as compared to the control group with no significance among different levels of HA. However, Herzig *et al.* (2009) observed that humic acid did not alter the daily weight gains of broiler chickens. Results of present study are in accordance with Ozturk *et al.* (2010); Nagaraju *et al.* (2014) and Ozturk *et al.* (2014) who reported that the use of HA on daily basis showed positive effect on broilers growth performance. Seemingly, Arif *et al.* (2016) indicated that dietary HA improved body weight gain and feed efficiency. In this respect, ELnaggar and El-Kelawy (2018) and Salah *et al.* (2015) reported that HA supplemented to broiler diets improved body weight gain, and feed conversion ratio. Perhaps, humic acid leads to stabilize animal gut micro flora which result in improved nutrient absorption and weight gain (Pistova *et al.*, 2016) Moreover, Arafat *et al.* (2015) postulated that 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 in the drinking water of broiler chickens (Ozturk *et al.*, 2010) or in the diet (Rath

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et al., 2006 and Taklimi *et al.*, 2012). 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 due to better utilization of nutrients resulting in increased body weight (Lala *et al.*, 2016). From the studies of Taklimi *et al.* (2012), it is suggested that the advantages of humic substances are expected to be: 1) the capacity to make defensive layers over the epithelial mucosal film of the gut against the passage of toxic and other bacterial contaminated substances. 2) the ability to reduce the absorption of nitrates, fluorites and heavy metals, causing detoxification in the gut 3) increasing immune receptors in the gut lining to protect against pathogens and 4) promoting growth.

In this connection, ELnaggar and El-Kelawy (2018) showed the effect of feeding graded levels of humic acid, on FC and FCR of Sasso strain chicks. Sasso chicks fed basal diet supplemented with 0.1% of humic acid had significantly better FCR

Apparent digestibility of nutrients:

Data concerning the effects of HA on the apparent digestibility of the nutrients and ash retention of broilers are shown in Table 3. Basal diet supplemented with different levels of HA improved ($P \leq 0.05$) both CP and EE digestibility compared to control basal diet. However, there were no significant effects of different levels of HA on crude fiber (CF), Dry matter digestibility and apparent ash retention.

The improvements in the apparent digestibility of the nutrients with humic acid in diet were obtained by Sheikh *et al.* (2010). In this respect, Taklimi *et al.* (2012) stated that supplementing humic acid substances to broiler diet increased the length villi which results in improved digestibility due to

lowering of the passage rate of the intestinal content and increasing the activity of digestive enzymes. They added that HA has the capacity to reduce the pH of the digestive tract causing decrease in metabolic needs and increase metabolism of protein and carbohydrates, thereby increasing the availability of nutrients. The same authors added that HA may stabilises the intestinal microflora and thus improved utilization of nutrients (ELnaggar and El-Kelawy, 2018)

Blood Biochemical parameters

The blood serum protein of broiler fed diet supplemented with different levels of HA at 39 day of age are shown in Table 4. HA supplementation gave significantly higher total protein, globulin and β - and γ -globulin in serum than the control group. However, HA supplementation did not significantly affect on albumin and Alb/Glo ratio of broiler at 39 day of age. In this connection, Šamudovská and Demeterová (2010) reported that fed diets supplemented with natural humic compounds (HS) and sodium humate (HNa) improved total protein. However, Avci *et al.* (2007) reported that no significant differences in serum total protein was observed for chicks received HA compared with the control group. Moreover, Can and Sakir (2009) confirmed that supplementation of 2.5 kg HA/ ton diet caused no statistical difference in serum total protein of broilers.

Glucose and thyroid hormones of blood serum of broiler fed diet supplemented with different levels of HA at 39 day of age are shown in Table 5. HA supplementation significantly increased blood glucose compared to the control group. Also, groups having HA gave significantly higher T_3 and T_4 concentrations in blood than the control. While, T_3/T_4 ratio did not significantly affected by HA supplementation to broiler diet at 39 day of age. With respect to blood glucose, Rath

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et al. (2006) revealed that there was a trend for decrease in glucose in broiler chickens of humic acid (HA). However, Šamudovská and Demeterová (2010) reported that when chickens were fed diets supplemented with humic compounds, higher value of glucose was observed at 35 days of age compared to control group.

Data concerning the effects of HA on the blood serum lipid profile of broiler at 39 day of age are shown in Table 6. Additions of different levels of HA to the feed had a significantly lower total lipids, cholesterol and LDL compared with the control group. While, HA supplementation did not have significant effect on the high-density lipoprotein (HDL) triglycerides (TG) and VLDL in blood serum. Data obtained are similar to Alena and Maria (2010) who showed that significantly lower concentration of cholesterol ($P < 0.05$) was observed in the sodium humate groups compared with the control

In contrast, Rath *et al.* (2006) reported that humic acid had no effect on cholesterol and triglycerides when humic acid was supplemented in water to broiler chicks. Also, Can and Sakir (2009) confirmed that supplementation of 2.5 kg HA /ton diet caused no statistical difference of serum cholesterol and triglycerides in broilers. Avci *et al.* (2007) investigated the effect of humic acid supplementation on Japanese quails and no effect had been reported on triglycerides and VLDL. While, ELnaggar and El-Kelawy (2018) with sasso chickens, showed that humic acid decreased serum total lipids, triglycerides, cholesterol, HDL and LDL compared to control group.

The blood serum antioxidant enzymes of broiler fed diet supplemented with different levels of HA at 39 day of age are listed in Table 7. Chicks fed basal diet supplemented with HA significantly increased TAC activity, superoxide dismutase (SOD) GPX activity and GSH activity while, decreased Malondialdehyde

(MDA) compared to the control. The same results were observed by ELnaggar and El-Kelawy (2018)

Carcass characteristics :

Relative weight of carcass characteristics and body organs of broiler chicks fed HA at 39 day of age are shown in Table 8. The different levels of HA significantly affected dressing percentage, spleen and thymus. In this connection, Mirnawati and Marida (2013) and Abdel-Mageed (2012) obtained higher ($P \leq 0.05$) values of dressing, breast and thighs % and lower ($P \leq 0.05$) abdominal fat % as compared to those fed control diet.

Results reported herein are consistent with the findings of Rath *et al.* (2006) who, found that the relative weights of the bursa of fabricius increased in birds given 0.25% humate suggesting a possible immunostimulatory impact of humate. Humic acid may exert a beneficial impact on immune systems of birds. The increase of relative weight of spleen and bursa of fabricius as result of humic acid addition could play a role in improving the immune function. Results obtained are also contradictory to Avci *et al.* (2007) who reported that no significant differences in slaughter characteristics were found between birds fed diet with humic acid compared with the control group. While, ELnaggar and Elkelawy (2018) found that addition of 0.1 and 0.2% of humic acid increased significantly that of dressing and decreased percentage of abdominal fat, compared to control

CONCLUSION

Dietary supplementation of HA at 1, 2, and 4 g/ kg diet improved productive performance, some blood parameters, carcass characteristic antioxidant status and immune response of broiler chicks.

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Table (1): Composition and calculated analysis of basal diets used in experiment (%)

Diets	Starter period (1-21d)	Grower period (22-39 d)
Ingredients,%		
Yellow Corn	54.00	59.00
Soybean Meal (46%)	27.00	21.20
Full fat soya,	5.00	7.00
Corn Glutein meal 60%	8.00	7.00
Soya oil	1.50	1.30
Mono calcium Phosphate	1.65	1.65
Lime stone	1.75	1.75
L-lysine HCL	0.25	0.25
DL –methionine	0.20	0.20
Salt (Na Cl)	0.35	0.35
Premix *	0.30	0.30
Total	100	100
Calculated analysis		
Crude protein %	23	21
ME (kcal/kg)	3050	3100
Crude Fiber, %	2.70	2.70
Ether extract, %	4.10	4.45
Calcium, %	1.01	1.01
Phosphorus available%	0.50	0.51
Methionine %	0.66	0.61
Lysine %	1.33	1.25
Methionine+Cystine %	1.05	0.98

*: Each kg of vitamin and mineral mixture contains: 12 M IU vitamin A; 5 M IU D₃; 80000 mg E; 4000 K mg; 4000 mg B₁; 9000 mg B₂; 4000 mg B₆; 20 mg B₁₂; 15000 mg pantothenic acid; 60000 mg Nicotinic acid; 2000 mg Folic acid; 150 mg Biotin; 400000 mg Choline Chloride; 15000 mg Copper sulphate; 1000 mg calcium Iodide; 40000 mg ferrous sulphate ; 100000 mg Manganese oxide ; 100000 mg Zinc oxide and 300 mg Selenium selenite.

Table (2): Effect of supplementation with different levels of humic acid on productive performance of broiler chicks

Dietary supplementations	LBW 7 d	LBW 39 d.	BWG 7-39 d	FC (7-39) d	FCR (7-39) d	EE	EPEI
Control	200.83	1820.44 ^b	1619.61 ^b	3233.34	2.00 ^a	0.610 ^c	233.33 ^c
Humic acid (1g/kg)	201.12	2210.22 ^a	2009.11 ^a	3298.89	1.64 ^b	0.929 ^b	345.53 ^a
Humic acid (2g/kg)	201.56	2229.22 ^a	2027.66 ^a	3279.66	1.62 ^b	0.961 ^a	351.38 ^a
Humic acid (3g/kg)	199.944	2100.22 ^a	1900.27 ^a	3143.89	1.65 ^b	0.921 ^b	326.34 ^b
Humic acid (4g/kg)	201.38	2180.44 ^a	1979.06 ^a	3236.28	1.64 ^b	0.936 ^b	340.84 ^b
SEM	1.90	21.80	19.98	10.98	0.045	0.980	4.09
<i>P value</i>	0.0951	0.0014	0.0020	0.0937	0.001	0.003	0.001

^{a,b,c} Means in the same column followed by different letters are significantly different at ($P \leq 0.05$); SEM, Standard error of mean. LBW= Live body weight BWG= Body weight gain , FC= Feed consumption, FCR= Feed conversion ratio, EE= Economic efficiency, EPEI= European production efficiency index

Table (3): Effect of different levels of humic acid supplementation on the apparent digestibility of the nutrients and ash retention of broiler chicks.

Dietary supplementations	Crude protein	Ether extract	Crude fiber	Dry matter	Apparent Ash retention,%
Control	63.87 ^b	66.13 ^b	16.00	64.90	30.00
Humic acid (1g/kg)	68.00 ^a	69.70 ^a	17.20	66.30	32.90
Humic acid (2g/kg)	71.12 ^a	70.81 ^a	16.88	65.11	33.48
Humic acid (3g/kg)	70.90 ^a	71.11 ^a	16.89	64.90	31.93
Humic acid (4g/kg)	67.30 ^{ab}	69.89 ^{ab}	17.01	63.76	29.02
SEM	2.90	2.90	1.99	1.88	2.09
<i>P value</i>	0.001	0.002	0.067	0.034	0.076

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

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Table (4): Effect of supplementation with different levels of humic acid on protein profile (g/dl) of broiler chicks.

Dietary supplementations	Blood protein profile (g/dl)						
	Total protein (g/dl)	Albumin (g/dl)	Globulin (g/dl)	A/G ratio	α -globulin (μ g/dl)	β -globulin (μ g/dl)	γ -globulin (μ g/dl)
Control	5.26 ^b	2.98	2.28 ^b	1.300	0.996	0.481 ^b	0.806 ^b
Humic acid (1g/kg)	6.36 ^a	3.22	3.14 ^a	1.024	0.73	0.721 ^a	1.69 ^a
Humic acid (2g/kg)	6.20 ^a	3.14	3.05 ^a	1.026	0.763	0.633 ^a	1.66 ^a
Humic acid (3g/kg)	6.10 ^a	2.92	3.18 ^a	0.918	0.63	0.742 ^a	1.81 ^a
Humic acid (4g/kg)	6.33 ^a	3.08	3.24 ^a	0.950	0.693	0.743 ^a	1.81 ^a
SEM	0.087	0.911	0.087	0.006	0.008	0.001	0.0056
P value	0.001	0.090	0.0002	0.0065	0.630	0.002	0.0010

a,b,c Means in the same column followed by different letters are significantly different at $P \leq 0.05$. SEM; Standard error of mean.

Table (5): Effect of supplementation with different levels of humic acid on blood glucose and thyroid hormones of broiler chicks.

Dietary supplementations	Blood biochemical parameters			
	Glucose (mg/dl)	T3 (ng/dl)	T4 (ng/dl)	T3 / T4 ratio
Control	180.11 ^b	3.07 ^b	8.96 ^b	0.343
Humic acid(1g/kg)	236.33 ^a	4.23 ^a	12.50 ^a	0.338
Humic acid(2g/kg)	222.00 ^a	4.07 ^a	12.03 ^a	0.338
Humic acid(3g/kg)	229.00 ^a	4.14 ^a	12.11 ^a	0.342
Humic acid(4g/kg)	234.67 ^a	4.16 ^a	12.35 ^a	0.337
SEM	3.98	0.986	0.876	0.006
P value	0.002	0.8105	0.0010	0.076

^{a,b}Means in the same column followed by different superscripts are significantly different at ($P \leq 0.05$); SEM= Standard error of means. T3= triiodothyronine; T4=thyroxine;

Table (6): Effect of supplementation with different levels of humic acid on indicators of antioxidative status of broiler chicks.

Dietary supplementations	Indicators of antioxidative status in blood (mg/dl)				
	TAC	GSH	GPX	SOD	MAD
Control	265.67 ^b	703.66 ^b	29.99 ^b	310.33 ^c	190.63 ^a
Humic acid(1g/kg)	378.33 ^a	826.33 ^a	37.91 ^a	342.67 ^b	137.33 ^c
Humic acid (2g/kg)	367.53 ^a	866.35 ^a	39.99 ^a	370.53 ^a	152.90 ^b
Humic acid (3g/kg)	366.67 ^a	890.00 ^a	38.89 ^a	369.67 ^a	156.47 ^b
Humic acid (4g/kg)	375.00 ^a	899.66 ^a	39.90 ^a	388.53 ^a	153.87 ^b
SEM	1.98	8.99	2.99	11.99	9.99
P value	0.001	0.0002	0.001	0.0447	0.004

^{a,b}Means in the same column followed by different letters are significantly different at (P ≤ 0.05); SEM, Standard error of mean. TAC=total antioxidant capacity; ; GSH-Px =glutathione peroxidase; SOD=superoxide dismutase, MDA= malondialdehyde

Table (7): Effect of supplementation with different levels of humic acid on lipids profile (mg/dl) of broiler chicks.

Dietary supplementations	Lipids profile (mg/dl)					
	T. Lipid	Chol.	TG.	HDL	LDL	VLDL
Control	490.88 ^a	196.67 ^a	80.67	50.47	130.06 ^a	16.13
Humic acid(1g/kg)	300.65 ^c	146.33 ^b	80.33	57.90	72.36 ^b	16.07
Humic acid (2g/kg)	310.11 ^c	156.00 ^b	88.33	52.07	86.26 ^b	17.67
Humic acid (3g/kg)	299.23 ^c	144.00 ^b	89.67	40.20	85.86 ^b	17.93
Humic acid (4g/kg)	360.45 ^b	135.33 ^b	86.33	44.37	73.71 ^b	17.27
SEM	2.90	11.00	2.98	4.11	8.09	1.99
P value	0.0005	0.0001	0.075	0.071	0.0005	0.098

^{a,b,c} Means in the same column followed by different superscripts are significantly different at(P≤ 0.05); SEM= Standard error of means, Chol.= total cholesterol; TG= triglycerides; HDL=high-density lipoprotein; LDL=low-density lipoprotein,VLDL= very low density lipoprotein

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Table (8): Effect of supplementation with different levels of humic acid carcass traits of broiler chicks.

Dietary supplementations	Carcass traits (%)				
	Carcass	Abdominal fat	Spleen	Thymus	Bursa
Control	66.44 ^b	0.65 ^a	0.131 ^b	0.267 ^c	0.081
Humic acid (1g/kg)	73.22 ^a	0.47 ^b	0.155 ^a	0.412 ^a	0.118
Humic acid (2g/kg)	72.60 ^a	0.36 ^b	0.200 ^a	0.409 ^a	0.121
Humic acid (3g/kg)	72.43 ^a	0.34 ^b	0.170 ^a	0.368 ^b	0.145
Humic acid (4g/kg)	68.23 ^{ab}	0.21 ^b	0.180 ^a	0.354 ^b	0.1.61
SEM	1.752	0.102	0.101	0.041	0.034
P value	0.001	0.001	0.004	0.009	0.067

^{a,b}. Means in the same column followed by different superscripts are significantly different at (P< 0.05); SEM= Standard error of mean.

REFERENCES

- Abdel-Mageed, M.A.A. 2012.** Effect of dietary humic substances supplementation on performance and immunity of Japanese quail. Egypt. Poultry Sci., 32: 645-660.
- Abou-Raya, A. K. and Galal, A. G. 1971.** Evaluation of poultry feeds in digestion trials with reference to some factors involved. J Anim Prod United Arab Repub.
- Alena, S. and D. Mária 2010.** Effect of Diet Supplemented with Natural Humic Compounds and Sodium Humate on Performance and Selected Metabolic Variables in Broiler Chickens ACTA VET. BRNO 79: 385–393.
- AOAC, 2004.** Official methods of analysis. 18th ed., Association of Official Analytical Chemists, Washington, DC, USA.
- Arafat, Y. Rana; H. K. Sohail; A. Javid and I. Ghulam 2015.** Effect of dietary humic acid via drinking water on the performance and egg quality of commercial layers. American Journal of Biology and Life Sciences., 3: 26-30.
- Arif, M., A. Rehman, M. Saeed, M. E.AbdEl-Hack, M.A. Arain, M.Haseebarshad, H. M.Zakria and I.M.Abbasi, 2016.** Impacts of dietary humic acid supplementation on growth performance, some blood metabolites and carcass traits of broiler chicks. Indian Journal of Animal Sciences 86 (9):1073–1078
- Avci, M.; N. Denek and O. Kaplan 2007.** Effects of Humic Acid at Different Levels on Growth Performance, Carcass Yield and Some Biochemical Parameters of Quails. Journal of Animal and Veterinary Advances, 6:1-4.
- Bauer, J.D. 1982.** Clinical laboratory methods, 9th edition, pp. 580–581. CV Mosby Co, USA.
- Bogin, E. and P. Keller 1987.** Application of clinical biochemistry of medically relevant animal models and standardization and quality control in animal biochemistry. Journal Clinical Chemistry Clinical Biochemistry, 25: 873-878.
- Bossuyt, X., Lissoir, B., Mariën, G., Maisin, D., Vunckx, J., Blanckaert, N., and Wallemacq, P. (2003).** Automated serum protein

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- electrophoresis by Capillarys®. *Clin Chem Lab Med*; 41(5):704–710.
- Can, A.K. and D. T. Sakir 2009.** The effect of humates on fattening performance, carcass quality and some blood parameters of broilers. *Journal of animal and veterinary Advances.*, 8:281-284.
- Doumas, B. 1971.** Colorimetric determination of serum albumin. *Clin. Chim. Acta*31: 400-403.
- Duncan, D.B. 1955.** Multiple range and multiple F tests. *Biometrics*, 11: 1–42.
- ELnaggar, A. S., & El-Kelawy, M. I. 2018.** Effect of humic acid supplementation on productive performance, blood constituents, immune response and carcass characteristics of sasso chicken. *Egyptian Journal of Animal Production*, 55(1), 75-84.
- Fossati, P., and Prencipe, L. 1982.** Serum triglycerides determined colorimetrically with an enzyme that produces hydrogen peroxide. *Clinical chemistry*, 28(10), 2077-2080.
- Friedewald, W. T., Levy, R. I., & Fredrickson, D. S. 1972.** Estimation of the concentration of low-density lipoprotein cholesterol in plasma, without use of the preparative ultracentrifuge. *Clinical chemistry*, 18(6), 499-502.
- Griggs, J.P. and J.P. Jacop 2005.** Alternatives to antibiotics for organic poultry production. *J. Apple. Poult. Res.*, 14: 750-756.
- Hayirli, A.; N. Esenbuga; M. Macit; E. Lacin; M. Karaoglu; H. Karaca and L. Yildiz 2005.** Nutrition practice to alleviate the adverse effects of stress on laying performance, metabolic profile, and egg quality in peak producing hens: I. The humate supplementation. *Asian-Aust. J. Anim. Sci.*, 18: 1310-1319.
- Henry, R.; Cannon, D. and Winkelman, J. 1974.** *Clinical chemistry, principles and techniques*,
- Herzig I.; M. Navratilova; J. Totusek; P. Suchy; V. Vecerek; J. Blahova and Z. Zraly 2009.** The effect of humic acid on zinc accumulation in chicken broiler tissues. *Czech J. Anim. Sci.*, 54: 121–127.
- Hubbard, S. A. S. 1999.** *Broiler management guide. Hubbard SAS, Quintin, France.*
- Islam, K.M.; S.A. Schuhmacher and M.J. Gropp 2005.** Humic acid substances in animal agriculture. *Pakistan J. Nutr.*, 4: 126–134.
- Joone G.K. and C.E.J. van Rensburg 2003.** Investigation of the immunostimulatory properties of oxihumate. *Z. Naturforsch*, 58: 263–267.
- Koracevic, D.; Koracevic, G.; Djordjevic, V.; Andrejevic, S. & Cosic, V. 2001.** Method for the measurement of antioxidant activity in human fluids. *Journal of clinical pathology*, 54(5), 356-361.
- Lala, A. O., N. Okwelum, K.O. Bello, N. A. Famakinde and M. O. Alamu, 2016.** Comparative study between ISA brown and fulani ecotype chickens supplemented with humic acid. *Slovak Journal of Animal Science*, 49 (2): 68–75
- Levy, S. 2014.** Reduced antibiotic use in livestock: How Denmark tackled
- Lopez-Virella, M. F. 1977.** Colorimetric method for determination of HDL-cholesterol. *Clin. Chem*, 23, 882.
- Mirnawati, Y.R. and Y. Marida, 2013.** Effects of humic acid addition via drinking water on the performance of broilers fed diets containing fermented

Broiler, Humic acid, Growth, Digestibility, Blood .

- and non-fermented palm kernel cake. *Archiva Zootechnica*, 16 (1): 41-53.
- Misra, H. P., & Fridovich, I. 1972.** The generation of superoxide radical during the autoxidation of hemoglobin. *Journal of Biological Chemistry*, 247(21), 6960-6962.
- Mudroňová, D., Karaffová, V., Pešulová, T., Koščová, J., Maruščáková, I. C., Bartkovský, M., ... & Marcinčák, S. 2020.** The effect of humic substances on gut microbiota and immune response of broilers. *Food and Agricultural Immunology*, 31(1), 137-149.
- Nagaraju, R., B. S. Reddy, R. Gloridoss, B.N. Suresh, C., Ramesh, 2014.** Effect of dietary supplementation of humic acids on performance of broilers, *Indian Journal of Animal Sciences*, 84(4): 447–452.
- Ozturk E.; N. Ocak; A. Turan; G. Erener; A. Altop and S. Cankaya 2012.** Performance, carcass, gastrointestinal tract and meat quality traits, and selected blood parameters of broilers fed diets supplemented with humic substances. *J. Sci. Food. Agric.*, 92: 59–65.
- Ozturk E.; N. Ocak; I. Coskun; S. Turhan and G. Erener 2010.** Effects of humic substances supplementation provided through drinking water on performance, carcass traits and meat quality of broilers. *J. Anim .Physiol. Anim. Nutr.*, 94:78–85.
- Ozturk, E., I. Coskun, N. Ocak, G. Erener, M. Dervisoglu and S. Turhan, 2014.** Performance, meat quality, meat mineral contents and caecal microbial population responses to humic substances administered in drinking water in broilers, *British Poultry Science*, 55(5): 668-674.
- Pistová, V., Arpášová, H., Hrněár, C., Kačaniová, M., & Haščík, P. 2016.** The Effect of the Humic Acid and Herbal Additive Supplement on Production Parameters of Broiler Chicken. *Scientific Papers: Animal Science & Biotechnologies/Lucrari Stiintifice: Zootehnie si Biotehologii*, 49(2).
- Placer, Z. A., Cushman, L. L., & Johnson, B. C. 1966.** Estimation of product of lipid peroxidation (malonyl dialdehyde) in biochemical systems. *Analytical biochemistry*, 16(2), 359-364.
- Rath N.C.; W.E. Huff and G.R. Huff 2006.** Effects of humic acid on broiler chickens. *Poultry Sci.*, 85: 410–414. resistance. *Environ Health Perspectives*, 122, A160–A165.
- Salah, Hala, M. El Sayed, R. R. Reham and Eman S. Abd El Hamid, 2015.** Study on the Effect of Humic Acid on Growth Performance, Immunological, Some Blood Parameters and Control Intestinal Closterdium in Broiler Chickens. *Zag. Veterinary J.*, 43(1): 102-109
- Šamudovská A. and M. Demeterová 2010.** Effect of Diet Supplemented with Natural Humic Compounds and Sodium Humate on Performance and Selected Metabolic
- SAS, (2006).** SAS/STAT User's guide statistics. SAS institute INC., Cary. NC, USA.
- Sheikh, A., B. Tufail, A. B. Gulam, S. M. Masood and R. Manzoor, 2010.** Effect of Dietary Supplementation of Organic Acids on Performance, Intestinal Histomorphology, and Serum Biochemistry of Broiler Chicken. *Veterinary Medicine International*, 2010, Article ID 479485,

- 7 pages
http://dx.doi.org.10.4061/2010/479485
- Simsek, N., Karadeniz, A., & Karaca, T. 2007.** Effects of the Spirulina platensis and Panax ginseng oral supplementation on peripheral. Revue Méd. Vét, 158(10), 483-488.
- Taklimi, S. M., Ghahri, H., & Isakan, M. A. 2012.** Influence of different levels of humic acid and esterified glucomannan on growth performance and intestinal morphology of broiler chickens. Agricultural Sciences, 3, 663-668. doi:10.4236/as.2012.35080
- Trinder, P. 1969.** Colorimetric method for the determination of blood glucose. Animal Climate Biochemistry, 6: 24. www.info@micro-plus.com.
- Trinder, P. (1969)** .Enzymatic colorimetric determination of glucose in serum, plasma or urine. Ann. of Clin. Biochem. 6: 24-26.
- Windisch, W. M.; K. Schedle; C. Pitzner and A. Kroismayr 2008.** Use of phytogenic products as feed additives for swine and poultry. J. of Anim. Sci., 86: E140-E148.
- Yasar, S.; A. Gokcimen; I. Altunas; Z. Yonden and E. Petekkaya 2002.** Performance and ideal histomorphology of rats treated with humic acid preparations. J. of Anim. Nutr., 86: 257-264.
- Zeweil, H. S., 1996.** Enzyme supplements to diets growing Japanese quails. Egyptian Poultry Science, 16: 535-557.

الملخص العربي

الاستجابة الانتاجية والفسيوولوجية لكتاكتيت التسمين لاضافه حمض الهيوميك في العليقه

عبد الله علي غزاله^١ - وليد صلاح الطحاوي^٢ - أسماء عبد العليم غلوش^٢ ؛ أسماء شوقي النجار^٢

١- قسم الإنتاج الحيواني - كلية الزراعة - جامعة القاهرة- مصر

٢- قسم الإنتاج الحيواني والداجني - كلية الزراعة - جامعة دمنهور- مصر

أجريت هذه الدراسة لتقييم تأثير اضافة حمض الهيوميك على الاداء الانتاجي لكتاكتيت التسمين. عند عمر أسبوع تم توزيع عدد مائة وثمانون كتكوت تسمين اربوايكرزالي خمسه مجموعات تجريبية بكل مجموعه ٣٦ طائر موزعه في ستة مكررات). غذيت المجموعة الأولى على العليقة الأساسية بدون اي اضافة (مجموعة كنترول)، أما المجموعات الثانية والثالثة والرابعة والخامسة فقد تغذت على العليقة الأساسية مضافا إليها ١-٢-٣-٤ جم من حمض الهيوميك / كجم علف. في نهاية التجربة تم أخذ عينات الدم لتحديد بعض مكونات سيرم الدم. ثم اجريت اختبارات الذبح باستخدام عدد ستة طيور من كل مجموعه .

أظهرت النتائج أن كتاكتيت التسمين المغذاه على ١-٢ جم من حامض الهيوميك سجلت أفضل أداء إنتاجي و كفاءة اقتصادية مقارنة بمجموعة الكنترول. ادي استخدام حمض الهيوميك بمستويات ١ و٢ و٣ جم /كجم علف الي زيادة معنويه في معامل هضم البروتين والدهن مقارنة بالكنترول. أدت إضافة حمض الهيوميك إلى خفض الدهون الكلية و الكولسترول منخفض الكثافة وكذلك زيادة محتوى بلازما الدم من الجلوكوز النثيروكسين و نشاط انزيمات الأكسدة GPX و SOD والبروتين الكلي والجاما الجلوبيولين و مقارنة بمجموعة الكنترول .

الخلاصه: ادي إضافة حمض الهيوميك بمستوي ١-٢-٣-٤ جم /كجم علف إلى تحسن أداء النمو، ومعاملات هضم ، ودليل الإنتاج والكفاءة الاقتصادية دون أي آثار سلبية على مكونات الدم وخصائص الذبيحه في كتاكتيت التسمين.