

THE EFFECT OF DIETRY HUMIC ACID SUPPLEMENTATION ON SOME PRODUCTIVE AND PHYSIOLOGICAL TRAITS OF LAYING HENS

By

Maysa, M. Hanafy and A. M. H. El-Sheikh

Poult. Bre. Res. Dept.; Anim. Prod. Res. Instit.; Minis. of Agric.; Giza; Egypt

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ABSTRACT: *This study was designed to investigate the effect of addition humic acid to the developed laying hen diets on egg production, egg quality and some physiological traits. At 20 weeks of age one hundred and twenty hens of Gimmizah strain were divided at random into three equal groups and represented 40 hens for each one. The first group was fed on a basal diet and served as control, the second and third groups were fed on a basal diets supplemented with 100 mg or 200 mg humic acid /Kg diet, respectively for a period of 24 weeks. The results were summarized as follow:*

- *Live body weight and feed consumption were not significantly affected by humic acid supplementation, while live body weight was significantly ($P \leq 0.05$) increased by developing hens age.*
- *Supplementation of humic acid to the laying hen diet caused significant ($P \leq 0.05$) increase in egg weight and egg production percentage compared with the control group. Also, these traits were significantly ($P \leq 0.05$) increased as the hen age increased. While the age at the first egg was significantly ($P \leq 0.05$) decreased in the groups fed humic acid, this reduction is related to the decrease of first egg weight. Therefore, addition of humic acid to laying hen diet could induce precocious sexual puberty.*
- *No significant effect of humic acid supplementation on egg shape index, albumin (%), Haugh unit and egg yolk index. These traits were not significantly affected by hen's age. Whereas, egg shell weight percentage and shell thickness were significantly ($P \leq 0.05$) increased compared with the control group. Moreover, shell thickness significantly ($P \leq 0.05$) increased at 32, 38 and 44 weeks of age.*
- *Addition of humic acid (especially with high level) to the laying hen diets caused a significant ($P \leq 0.05$) increase in RBC`s, WBC`s, hemoglobin, plasma calcium and total protein compared with other groups. The highest values of WBC`s, hemoglobin and plasma total protein were observed at 44*

weeks of age. While plasma albumin, GOT, GPT and T₃ concentrations did not significantly affected by humic acid supplementation or the hen's age.

- *Relative weights of spleen, ovary and oviduct length were significantly ($P \leq 0.05$) increased with higher dose of humic acid compared with the control. Whereas, humic acid supplementation had no significant effect on relative weights of carcass, liver, gizzard, heart and oviduct.*

It can be concluded that supplementation of humic acid to laying hen diet especially with the higher dose (200 mg/ Kg diet) can be used to improve egg production traits, shell quality, some physiological and immunity traits.

INTRODUCTION

Humic acid (HA) is resulting from decomposition of organic matter, particularly plants, and it is natural components of drinking water, soil and lignite, moreover, it has been used as an antidiarrheal, analgesic, immunostimulatory, and antimicrobial agent in veterinary practices in Europe (EMEA, 1999). Many experimental studies have shown HA to be nontoxic and nonteratogenic (EMEA, 1999 and Yasar *et al.*, 2002).

Humates are the salts of humic acid in which the exchange site is Ca⁺, Na⁺, Al⁺, and Fe⁺² rather than hydrogen (HuminTech, 2004). The idea of using humates in animal nutrition is recent. At first, humates increased feed conversion efficiency in calves, dogs, and cats and used as a part of replacement therapy for digestive system disturbances such as malnutrition and diarrhea (Kuhnert *et al.*, 1989, 1991). Remarkable changes in electrolyte balance and enhancements in immune potency in response to humate supplementation have been reported in ruminants (Lenk and Benda, 1989 and Griban *et al.*, 1991) and in poultry (Parks *et al.*, 1996). Moreover, consistent agreements in the limited number of published articles show that humates promote growth by altering partitioning of nutrient metabolism (Stepchenko *et al.*, 1991; Zhorina and Stepchenko, 1991 and Parks, 1998), improve feed conversion efficiency (Shermer *et al.*, 1998), increase egg production (Yoruk *et al.*, 2004) and improve egg weight (Kucukersan *et al.*, 2005). Therefore, this research was carried out to investigate the effects of dietary humic acid supplementation on some productive, physiological, immunity and carcass traits of laying hens.

MATERIALS AND METHODS

Birds, Diet and Management

The study was carried out at El-Sabahia Poultry Research Station (Alexandria), Animal production Research Institute, Agricultural Research Center, Ministry of Agriculture. A total of 120 hens from Gimmizah strain at 20 weeks of age was used and randomly assigned in layer cages for three equal experimental groups. Hens were fed a basal diet of layers or the basal diet supplemented with either 100 mg humic acid (HA₁₀₀) or 200 mg humic acid /Kg diet (HA₂₀₀) during the experimental period (24 weeks). Each kg of humic acid contained 85% polymeric polyhydroxy acid, 10% phosphorous, 2% magnesium, 2% sulphur and 1% trace minerals (iron, zinc and manganese). Hens were fed *ad libitum* on a layer diet (Table 1). Water was available all the times, and lighting program of 16 hours a day was applied.

Sample Collection and Analytical Procedure

Hen body weight by gram (every 6 weeks), feed consumption by g/hen/day (daily), egg weight by gram and egg production percentage (daily) were recorded during the experimental periods. At 26, 32, 38 and 44 weeks of age, blood samples were obtained in heparinized tubes from the brachial vein of randomly five birds in each group. At the first, red blood cells (RBC`s) count, white blood cells (WBC`s) count and hemoglobin (Hb) were determined. Then, blood samples were centrifuged at 3000 rpm for 15 minutes to separate clear plasma which was stored at – 20 °C for determination of calcium (Ca), total lipids (TL), total protein (TP), albumin (AP), glutamic oxaloacetic transaminase (GOT) and glutamic pyruvic transaminase (GPT) concentrations by spectrophotometer using available commercial Kits produced by Sentinel, Italy. Triiodothyronine (T3) was determined in plasma by using radioimmunoassay Kit. Ten eggs were randomly taken from each group at the time of blood sampling for egg quality measurements [shape index, albumen (%), Haugh units, yolk (%), yolk index, shell (%) and shell thickness (mm)]. At the end of the experimental period (44 weeks of age), five random hens from each group were sacrificed to calculate relative weight of carcass, liver, gizzard, heart, spleen, ovary and oviduct and oviduct length (cm).

Statistical Analysis

Data were statistically analyzed by the ANOVA using SAS software (SAS, 1990) and the means were compared by the Duncan's multiple-range test (Duncan, 1955).

RESULTS AND DISCUSSION

Live Body Weight

Results in Table 2 indicated that humic acid supplementations had no significant effect on live body weight through all ages of the experiment. Therefore, overall mean of live body weight was not significantly affected by humic acid supplementation. Whereas, live body weight was significantly ($P \leq 0.05$) increased with the increase of hens age, this increase is expected as a result of age increase. These results are in agreement with those reported by Kocabagli *et al.*, (2002) and Karaoglu *et al.*, (2004) who indicated that no significant effect on body weight and daily weight gain of broiler chickens fed diet with humate compared with the control group. While, Shermer *et al.*, (1998) showed that the humic acid stabilizes the intestinal microflora and thus ensures an improved utilization of nutrients in animal feed, this leads to an increase in the live body weight of laying hens.

Feed consumption and Egg Production Traits

Results presented in Table 2 indicated that no significant effect of humic acid levels on feed consumption (g/hen/day). Regardless of humic acid supplementation, overall mean of feed consumption was not significantly affected by the increase of laying hen age. Similar result was obtained by Yoruk *et al.*, (2004) who found that humate with concentration of 0.1 and 0.2 % had no significant effect on feed intake in late stage of laying. Also, in broiler chickens, Kocabagli *et al.*, (2002) indicated that no significant effect on feed consumption was observed when groups fed diet with humate. While, Kucukersan *et al.*, (2005) showed that the average daily feed consumption of hen fed diets with humic acid was significantly

($P \leq 0.05$) decreased compared with the control group. Also, Table 2 showed that either of the dietary supplementation level of humic acid had a significant effect on egg weight and egg production percentage during the experimental period (24 weeks). Moreover, humic acid at 100 mg or 200 mg /Kg diet caused a significant ($P \leq 0.05$) increase in egg weight by 7.5 % and 11.4% and egg production percentage by 7.7% and 16.7%, respectively compared with the control group. Regardless of humic acid supplementation, overall mean of egg weight was significantly ($P \leq 0.05$) increased at 44 weeks of age compared to those at 26, 32 and 38 weeks of age. Also, overall mean of egg production percentage was significantly ($P \leq 0.05$) increased at 38 and 44 weeks of age. Therefore, egg weight and egg production percentage were significantly ($P \leq 0.05$) increased as the hen age increased. Also, there are significant ($P \leq 0.05$) effect in the interaction between age of hens and humic acid supplementation during the

experimental period. These results are consistent with those reported by Kucukersan *et al.*, (2005) who showed that the dietary humic acid at doses of 30 and 60 g / ton feed can be used to improve egg weight and egg production. Yoruk *et al.*, (2004) found that supplementation of humate in layer diets at 0.1 and 0.2 % for 75 days during the late laying period caused egg production increase compared to control group. While, no significant effect on egg weight was observed. On the other hand, Wang *et al.*, (2007) indicated that the dietary humic substances at 5 or 10 % decreased egg production but egg weight was improved.

Results in Table 2 showed that the addition of humic acid at 100 or 200 / Kg diet significantly ($P \leq 0.05$) decreased the age at first egg. These reductions were 11 and 13 days, respectively compared with the control group. Therefore, addition of humic acid to laying hen diets could induce precocious sexual puberty. Addition of humic acid to laying hen diet especial high level (200 mg) caused a significant increase in plasma calcium concentration (Table 4) therefore; the high calcium concentration in the experimental groups can also be associated with early laying as explained by (Ertas *et al.*, 2006). Also Table 2 demonstrated that egg weight of first egg was numerically decreased with the increase of humic acid concentration compared with the control group. This notice of egg weight decrease could be due to the early sexual maturity for group fed humic acid levels.

Egg quality

It was observed that there were no significant differences among treatments with respect to egg shape index, albumen percentage, Haugh unit and yolk index (Table 3). These traits were not affected by addition of humic acid to layer hen diets among hen age periods. Regardless of humic acid supplementation, overall means of all previous mentioned traits were not significantly affected by hen age. The results of Yoruk *et al.*, (2004); Kucukersan *et al.*, (2005) and Wang *et al.*, (2007) support our findings regarding that there were no significant effects of humic acid supplementation on the studied egg quality traits. Overall means of egg yolk percentage significantly ($P \leq 0.05$) decreased for hens fed humic acid supplementations compared with control group. While, overall means of egg shell percentage and egg shell thickness significantly ($P \leq 0.05$) increased for hens fed either levels of humic acid supplementation compared with control group (Table 3). The increases in egg shell percentage was more pronounced when hens fed diet with high level of humic acid, whereas, no significant differences was noticed between humic acid levels on egg shell thickness. Irrespective of humic acid supplementation, overall means of egg

shell thickness significantly ($P \leq 0.05$) increased at 32, 38 and 44 weeks of ages compared to those at 26 weeks of age. The increase of egg shell percentage and egg shell thickness especially with higher dose of dietary humic acid in the present study could be due the increase of plasma calcium concentration as demonstrated in Table 4. These results approach with those reported by Wang *et al.*, (2007) who indicated that the egg shell breaking strength as indicator of shell thickness was increased for hen fed diets with humic substances compared with the control group. While, Kucukersan *et al.*, (2005) found that there were no changes in egg shell thickness and egg shell breaking strength in hens supplemented with humic acid.

Blood parameters

Overall means of RBC`s, WBC`s and Hb were significantly ($P \leq 0.05$) increased for hens fed humic acid supplementation compared with the control group (Table 4). Irrespective of humic acid supplementation, overall mean of age related changes in RBC`s had not significantly influenced, while, overall mean of WBC`s was significantly ($P \leq 0.05$) increased at 44 weeks of age compared to those at 26, 32 and 38 weeks of age. Also overall mean of Hb was significantly ($P \leq 0.05$) increased at 38 and 44 weeks of age compared to those at 26 and 32 weeks of age. Therefore, WBC`s and Hb significantly ($P \leq 0.05$) increased as the hen age increased. A similar result was obtained by Cetin *et al.*, (2006) who observed that the humate supplementation caused statistically significant increases ($p < 0.05$) in the erythrocyte count for laying hens. Also, Ipek *et al.*, (2008) found that RBC`s and Hb were significantly higher in groups fed humic acid compared with control group of Japanese quails. While, Rath *et al.*, (2006) and Ipek *et al.*, (2008) showed that humic acid did not have any effect on WBC`s in broiler chickens or Japanese quail, respectively.

Results presented in Table 4 indicated that overall means of plasma calcium and total protein concentrations significantly ($P \leq 0.05$) increased for hens fed high level of humic acid compared to other groups. Supported our results, Ertas *et al.*, (2006) who reported that humic acid improved protein digestion as well as calcium in Japanese quail, moreover, he added that high calcium concentration in the experimental groups can also be associated with early laying. Which add credence to our results related to early sexual mature as presented in Table 2. Also, Avci *et al.*, (2007) concluded that serum calcium concentrations were increased while serum total protein did not changed for hens fed diets with humic acid. Irrespective of humic acid supplementation, overall means of plasma calcium concentration was not influenced by the increase of laying hen age, while,

overall means of plasma TP significantly ($P \leq 0.05$) increased at 44 weeks of age compared to those at 26, 32 and 38 weeks of age.

Results in Table 5 showed that humic acid supplementation had no significant effect on plasma Ab, GOT, GPT and T_3 concentrations. Also, irrespective of humic acid supplementation, age- related changes in the previous mentioned traits were not significantly influenced. A similar conclusion was drawn by Van Rensburg *et al.*, (2006) who reported that insignificant differences on serum enzyme activity and albumin were observed among group fed diets with 2.3 g of oxihumate / Kg diet compared with control group. Whereas, Rath *et al.*, (2006) showed serum albumin concentration in broiler chicken was decreased in birds treated with humic acid.

Slaughter traits

Results presented in Table 6 showed that there were insignificant differences in relative weights of carcass, liver, gizzard, heart and oviduct between laying hens fed diet supplemented with 100 or 200 mg humic acid and those of the control group. Generally, there were some numerical increases in these traits with increase of humic acid supplementation. These results are in harmony with data obtained by Eren *et al.*, (2000); Kocabagli *et al.*, (2002) and Avci *et al.*, (2007) who reported that no significant differences in slaughter characteristics were observed between birds fed diet with humate or humic acid compared with the control group in broiler chickens or Japanese quails. On the other hand, results in Table 6 showed that relative weight of ovary and oviduct length were significantly ($P \leq 0.05$) increased for hens fed diet with 100 or 200 mg humic acid compared with the control group. Increase relative weight of ovary and oviduct length (cm) in the present experiment reflects and contributes in the increment of egg production for hens fed humic acid compared with hens fed control diet.

Also, 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 (Table 6). The results obtained from this study indicate that the increase of relative weight of spleen and white blood cells as result of humic acid supplementation could play a role in improving the immune function. These results approach with those reported by Rath *et al.*, (2006) and EMEA, (1999) who indicated that the relative weights of bursa of fabricius increased in chickens given 2.5 % humic acid suggesting a possible immunostimulatory effect that has been suggested to be an effect of humic acid. Moreover, Klocking *et al.*, (2002); Schepetkin *et al.*, (2003) and Joone

et al., (2003) showed that humic acid having immunostimulatory, anti-inflammatory and antiviral effects.

In conclusion, dietary humic acid supplementation especially with the higher dose of humic acid (200 mg/ Kg diet) could be used to improve egg production, shell quality, some physiological and immunity traits of laying hens. However, addition of different levels of humic acid to laying hens diet needs further investigations during different stages of egg production for complete evaluation.

Table 1: Composition and calculated analysis of basal diet.

Ingredients	%
Yellow corn	64.00
Soybean meal 44%	24.78
Wheat bran	1.00
Di-calcium phosphate	1.61
Limestone	7.91
DL-Methionine	0.10
Sodium chloride	0.30
Vit. & Min. Mixture*	0.30
Total	100.00
Calculated analysis:	
Metabolizable energy (Kcal/Kg)	2718.00
Crude protein %	16.02
Crude fiber %	3.46
Crude fat %	2.96
Calcium %	3.34
Available phosphorous %	0.42
Lysine %	0.89
Methionine %	0.39
Met+cystine %	0.66

*Supplied per kg diet: Vit A, 10000IU; Vit D₃, 2000 IU; Vit E, 10 mg; Vit K₃, 1 mg; Vit B₁, 1 mg; Vit B₂, 5mg; Vit B₆, 1.5 mg; Vit B₁₂, 10 mcg; Niacin, 30 mg; Pantothenic acid, 10 mg; Folic acid, 1 mg; Biotin, 50mcg; Choline, 260 mg; Copper, 4 mg; Iron, 30 mg; manganese, 60 mg; Zinc, 50 mg; Iodine, 1.3 mg; Selenium, 0.15mg; Cobalt, 0.1mg.

Table 2: Effect of dietary humic acid (HA) on layers performance at different ages (Means \pm SE).

Age (weeks)	Control	HA ₁₀₀	HA ₂₀₀	overall mean
Body weight (g)				
26	1491.43 \pm 29.93	1516.83 \pm 27.04	1543.52 \pm 31.36	1517.26 \pm 15.05 ^C
32	1598.53 \pm 37.22	1633.55 \pm 37.84	1655.63 \pm 31.28	1629.25 \pm 20.11 ^B
38	1752.33 \pm 38.07	1760.50 \pm 40.94	1789.95 \pm 30.30	1767.59 \pm 29.17 ^A
44	1758.3 \pm 35.04	1767.70 \pm 47.19	1797.9 \pm 35.11	1774.63 \pm 25.43 ^A
Overall mean	1650.15 \pm 30.24	1669.65 \pm 28.90	1696.75 \pm 20.31	
Feed consumption (g/ hen/ day)				
26	107.32 \pm 1.47	108.95 \pm 1.18	110.16 \pm 1.15	108.81 \pm 0.95
32	116.43 \pm 0.52	115.35 \pm 0.51	115.04 \pm 0.43	115.61 \pm 0.33
38	118.49 \pm 0.25	117.35 \pm 0.38	117.14 \pm 0.37	117.66 \pm 0.18
44	119.08 \pm 0.29	119.68 \pm 0.12	118.91 \pm 0.30	119.22 \pm 0.16
Overall mean	115.33 \pm 0.63	115.33 \pm 0.55	115.31 \pm 0.56	
Egg weight (g)				
26	37.69 \pm 0.48 ^j	40.33 \pm 0.42 ⁱ	42.41 \pm 0.46 ^h	40.14 \pm 0.23 ^D
32	40.32 \pm 0.66 ⁱ	45.86 \pm 0.52 ^g	47.63 \pm 0.42 ^{fg}	44.60 \pm 0.43 ^C
38	46.66 \pm 0.63 ^g	48.90 \pm 0.56 ^f	50.45 \pm 0.42 ^e	48.67 \pm 0.29 ^B
44	49.60 \pm 0.62 ^{ef}	52.33 \pm 0.55 ^d	53.63 \pm 0.48 ^d	51.85 \pm 0.22 ^A
Overall mean	43.57 \pm 1.01 ^c	46.85 \pm 0.98 ^b	48.53 \pm 0.89 ^a	
Egg production (%)				
26	31.08 \pm 1.23 ^h	34.61 \pm 1.30 ^h	40.30 \pm 1.46 ^g	25.33 \pm 0.90 ^C
32	60.71 \pm 1.79 ^f	58.89 \pm 1.54 ^f	67.86 \pm 1.60 ^e	52.51 \pm 1.20 ^B
38	62.86 \pm 1.31 ^f	68.57 \pm 1.39 ^{de}	68.93 \pm 1.34 ^{de}	56.79 \pm 0.85 ^A
44	59.10 \pm 1.43 ^f	68.10 \pm 1.19 ^e	72.44 \pm 1.62 ^d	56.54 \pm 0.76 ^A
Overall mean	53.44 \pm 2.20 ^c	57.56 \pm 4.02 ^b	62.38 \pm 3.90 ^a	
Age at first egg (day)				
	174.43 \pm 2.25 ^a	163.60 \pm 2.20 ^b	161.48 \pm 1.76 ^b	
Weight first egg (g)				
	40.64 \pm 0.75	39.34 \pm 0.72	38.74 \pm 0.70	

a,b,c,d,e,f,g = Means having different letters exponents within row are significant different ($P \leq 0.05$).

A, B, C,d = Means having different letters exponents within column are significant different ($P \leq 0.05$).

d,e,f,g,h,i,j = Means within age of hens by humic acid supplementation interaction effect within no common superscript differ significantly ($P \leq 0.05$).

Table 3: Effect of dietary humic acid (HA) on some egg quality at different ages. (Means \pm SE).

Age (weeks)	Control	HA ₁₀₀	HA ₂₀₀	overall mean
Egg shape index				
26	75.24 \pm 1.67	76.62 \pm 1.28	76.00 \pm 1.37	75.95 \pm 0.90
32	75.22 \pm 1.09	75.28 \pm 1.07	74.88 \pm 1.36	75.13 \pm 0.82
38	76.05 \pm 1.42	75.42 \pm 1.55	76.69 \pm 0.82	76.05 \pm 0.65
44	75.70 \pm 1.57	75.01 \pm 0.37	75.48 \pm 0.60	75.32 \pm 0.22
overall mean	75.55 \pm 1.00	75.58 \pm 0.89	75.76 \pm 0.73	
Egg albumen (%)				
26	56.32 \pm 1.38	56.48 \pm 1.74	55.34 \pm 0.81	56.05 \pm 0.70
32	56.86 \pm 0.59	55.05 \pm 3.50	54.99 \pm 0.87	55.63 \pm 1.23
38	56.66 \pm 1.70	56.03 \pm 1.56	54.69 \pm 0.68	55.79 \pm 0.49
44	56.55 \pm 0.88	55.84 \pm 0.73	55.66 \pm 0.84	56.02 \pm 0.33
overall mean	56.60 \pm 0.44	55.85 \pm 0.67	55.17 \pm 0.47	
Haugh unit				
26	90.06 \pm 1.12	86.11 \pm 1.18	87.95 \pm 1.65	88.04 \pm 0.77
32	89.34 \pm 3.29	91.91 \pm 1.46	90.83 \pm 1.37	90.69 \pm 1.01
38	86.40 \pm 2.24	87.40 \pm 2.55	88.10 \pm 3.20	87.30 \pm 1.85
44	89.94 \pm 1.40	89.86 \pm 2.66	90.28 \pm 2.17	90.03 \pm 1.11
overall mean	88.94 \pm 1.02	88.82 \pm 0.85	88.29 \pm 1.41	
Egg yolk (%)				
26	29.22 \pm 0.96	27.15 \pm 1.04	28.22 \pm 0.44	29.20 \pm 0.55
32	30.08 \pm 0.56	29.28 \pm 0.51	30.04 \pm 0.73	29.95 \pm 0.37
38	30.14 \pm 0.78	28.98 \pm 0.70	29.69 \pm 0.68	29.25 \pm 0.58
44	30.88 \pm 0.82	30.31 \pm 0.73	31.91 \pm 0.85	29.51 \pm 0.60
overall mean	31.34 \pm 0.25 ^a	29.26 \pm 0.32 ^b	29.58 \pm 0.18 ^b	
Egg yolk index				
26	45.01 \pm 1.49	44.04 \pm 1.27	44.74 \pm 0.51	44.60 \pm 0.66
32	45.33 \pm 0.88	46.08 \pm 0.65	45.87 \pm 0.78	45.76 \pm 0.38
38	44.21 \pm 0.37	43.80 \pm 0.86	43.76 \pm 0.79	43.92 \pm 0.26
44	45.25 \pm 0.82	45.55 \pm 1.11	46.02 \pm 1.77	45.61 \pm 0.96
overall mean	44.95 \pm 0.25	44.87 \pm 0.75	45.15 \pm 0.45	
Egg shell (%)				
26	12.46 \pm 0.83	14.37 \pm 0.72	15.44 \pm 0.57	14.76 \pm 0.67
32	12.06 \pm 0.24	15.22 \pm 0.50	14.97 \pm 0.52	14.08 \pm 0.68
38	12.17 \pm 1.05	14.99 \pm 0.91	15.62 \pm 0.35	14.26 \pm 0.80
44	11.57 \pm 0.91	14.86 \pm 0.67	15.99 \pm 0.33	14.81 \pm 0.82
overall mean	12.06 \pm 0.38 ^c	14.86 \pm 0.33 ^b	15.05 \pm 0.24 ^a	
Shell thickness (mm)				
26	0.362 \pm 0.03	0.426 \pm 0.02	0.446 \pm 0.01	0.413 \pm 0.02 ^B
32	0.424 \pm 0.02	0.490 \pm 0.02	0.528 \pm 0.02	0.487 \pm 0.02 ^A
38	0.416 \pm 0.01	0.481 \pm 0.01	0.486 \pm 0.01	0.461 \pm 0.01 ^A
44	0.428 \pm 0.02	0.486 \pm 0.01	0.495 \pm 0.01	0.470 \pm 0.01 ^A
overall mean	0.408 \pm 0.01 ^b	0.474 \pm 0.01 ^a	0.493 \pm 0.01 ^a	

a,b,c = Means having different letters exponents within row are significant different ($P \leq 0.05$).

A, B = Means having different letters exponents within column are significant different ($P \leq 0.05$).

Table 4: Effect of dietary humic acid (HA) on layers red blood cells (RBC's), white blood cells (WBC's), hemoglobin (Hb) and plasma calcium (Ca), total lipids (TL) and plasma total protein (TP) concentrations at different ages (Means ± SE).

Age (weeks)	Control	HA ₁₀₀	HA ₂₀₀	overall mean
RBC's (10 ⁶ /ML)				
26	1.19±0.35	2.04±0.29	3.00±0.47	2.08±0.32
32	1.11±0.11	1.89±0.16	2.59±0.21	1.86±0.25
38	1.54±0.33	2.08±0.34	2.72±0.26	2.11±0.19
44	1.36±0.14	1.72±0.16	2.18±0.04	1.75±0.12
overall mean	1.30±0.13 ^c	1.93±0.14 ^b	2.62±0.15 ^a	
WBC's (10 ⁶ /ML)				
26	10.29±1.23	12.53±3.33	16.16±1.35	13.00±1.48 ^{BC}
32	8.68±1.96	10.13±2.48	19.23±1.23	11.68±1.16 ^C
38	8.03±2.08	16.73±0.93	29.13±3.23	14.63±1.22 ^B
44	14.53±2.1	19.35±2.25	34.18±1.38	19.35±1.11 ^A
overall mean	10.38±1.01 ^c	14.69±0.81 ^b	18.93±1.12 ^a	
Hb (g/dl)				
26	8.54±0.82 ^e	9.35±0.86 ^e	10.14±0.31 ^e	9.34±0.25 ^B
32	9.0±0.17 ^e	9.55±0.19 ^e	11.92±0.78 ^{de}	10.16±0.11 ^B
38	10.86±1.20 ^e	16.32±0.65 ^d	17.61±0.76 ^d	14.76±0.55 ^A
44	10.76±0.44 ^e	15.05±1.59 ^d	18.54±2.73 ^d	14.78±0.35 ^A
overall mean	9.67±0.37 ^b	12.57±0.43 ^a	14.55±0.55 ^a	
Ca (mg/dl)				
26	11.61±1.76	10.73±1.29	16.30±1.13	12.88±1.00
32	11.65±0.94	13.03±0.46	18.24±2.00	14.30±1.03
38	12.24±0.51	14.13±2.10	17.78±1.00	14.72±0.96
44	11.69±1.02	14.25±0.97	16.91±0.91	14.18±0.64
overall mean	11.80±0.55 ^b	13.03±0.70 ^b	17.23±0.77 ^a	
TP (g/dl)				
26	5.84±0.40	5.40±0.33	5.87±0.29	5.70±0.17 ^B
32	4.52±0.14	4.64±0.23	4.93±0.19	4.70±0.09 ^C
38	5.17±0.08	5.69±0.13	6.84±0.30	5.90±0.11 ^B
44	6.48±0.36	7.21±0.22	7.51±0.27	7.07±0.16 ^A
overall mean	5.51±0.23 ^b	5.74±0.10 ^b	6.39±0.21 ^a	

a,b,c = Means having different letters exponents within row are significant different (P ≤ 0.05).

A, B, C = Means having different letters exponents within column are significant different (P ≤ 0.05).

d,e = Means within age of hens by humic acid supplementation interaction effect within no common superscript differ significantly (P ≤ 0.05).

Table 5: Effect of dietary humic acid (HA) on layers albumin (Ab), glutamic oxaloacetic transaminase (GOT) and glutamic pyruvic transaminase (GPT) and triiodothyronine (T₃) concentrations at different ages (Means ± SE).

Age (weeks)	Control	HA ₁₀₀	HA ₂₀₀	overall mean
Ab (g/dl)				
26	2.94±0.15	3.12±0.24	2.96±0.15	3.01±0.07
32	2.39±0.70	2.46±0.13	2.59±0.09	2.48±0.20
38	3.83±0.23	3.96±0.13	3.70±0.13	3.83±0.08
44	3.16±0.08	3.04±0.05	2.86±0.30	3.02±0.10
overall mean	3.08±0.21	3.15±0.16	3.03±0.13	
GOT (U/L)				
26	51.72±9.40	52.08±3.78	51.92±8.53	51.91±4.46
32	52.58±3.15	53.04±6.57	52.46±7.15	52.69±2.06
38	53.18±17.00	53.24±16.09	54.40±20.14	53.61±10.18
44	54.84±13.79	54.92±5.53	54.12±20.97	54.63±11.01
overall mean	53.08±8.16	53.32±5.13	53.23±11.17	
GPT (U/L)				
26	9.98±2.00	9.66±0.44	10.15±1.90	9.93±0.20
32	10.99±0.79	9.99±1.27	10.50±1.01	10.49±0.59
38	10.08±1.80	10.11±0.95	10.72±1.33	10.30±0.85
44	11.14±2.61	11.26±2.24	11.24±1.85	11.21±0.100
overall mean	10.55±1.01	10.26±1.11	10.65±0.65	
T ₃ (ng/dl)				
26	139.26±7.14	134.92±12.23	137.28±12.26	137.15±5.12
32	139.86±15.53	138.42±6.84	135.68±12.59	137.98±8.53
38	120.02±19.50	120.03±14.97	119.85±13.51	119.97±11.23
44	133.07±18.26	131.53±6.42	132.81±5.30	132.47±9.66
overall mean	133.05±6.66	131.05±9.90	131.40±8.91	

Table 6: Effect of dietary humic acid (HA) on relative weight of carcass, liver, gizzard, heart, spleen, ovary, oviduct and oviduct length (cm) of laying hens at 44 weeks of age (Means ± SE).

Parameter	Control	HA ₁₀₀	HA ₂₀₀
Carcass (%)	69.38±2.30	69.17±1.70	69.81±2.30
Liver (%)	2.21±0.13	2.28±0.19	2.30±0.02
Gizzard (%)	2.72±0.38	2.74±0.15	2.98±0.34
Heart (%)	0.45±0.03	0.46±0.08	0.48±0.02
Spleen (%)	0.14±0.03 ^b	0.19±0.02 ^{ab}	0.23±0.02 ^a
Ovary (%)	0.467±0.02 ^c	0.569±0.08 ^b	0.656±0.05 ^a
Oviduct (%)	2.19±0.47	2.97±0.23	3.03±0.15
Oviduct length (cm)	57.7±2.06 ^b	62.88±1.54 ^a	68.78±1.75 ^a

a, b, c = Means having different letters exponents within row are significant different (P ≤ 0.05).

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الملخص العربى

تأثير إضافة حمض الهيوميك للغذاء على بعض الصفات الإنتاجية والفسيوولوجية فى الدجاج البياض

مايسه مصطفى حنفى ، على محمد حسن الشيخ

قسم بحوث تربية الدواجن - معهد بحوث الإنتاج الحيوانى - وزارة الزراعة
- الجيزة - مصر

أجريت هذه التجربة لدراسة تأثير إضافة حمض الهيوميك لغذاء الدجاجات البيضاء المحلية خلال فترة إنتاج البيض من عمر 20 حتى 44 أسبوع على صفات إنتاج البيض و جودة البيض و بعض الصفات الفسيولوجية. حيث استخدم 120 دجاجة من سلالة الجميرة المحليه عمر 20 أسبوع قسمت إلى ثلاث مجموعات متساوية (40 دجاجة فى كل مجموعة). استخدمت المجموعة الأولى ككنترول و غذيت على العليقة الأساسية بينما غذيت كل من المجموعتين الثانية و الثالثة على العليقة الأساسية مضاف إليها 100 ملجم أو 200 ملجم حمض الهيوميك / كجم علف على الترتيب لمدة 24 أسبوع . و تلخص النتائج المتحصل عليها فيما يلى :

- ١ - إضافة حمض الهيوميك فى علف الدجاج لم يؤثر معنوياً على وزن الجسم الحى و الغذاء المستهلك بينما زاد وزن الجسم زيادة معنوية بزيادة عمر الدجاج.
 - ٢ - أدى إضافة حمض الهيوميك فى علف الدجاج البياض إلى زيادة معنوية لكل من وزن البيضة و النسبة المئوية لإنتاج البيض مقارنة بمجموعة الكنترول وكذلك زيادة تلك الصفات معنوياً بزيادة عمر الدجاج البياض. فى حين أنخفض معنوياً العمر عند وضع أول بيضة و صاحب ذلك انخفاض غير معنوى فى وزن أول بيضة مقارنة بمجموعة الكنترول و بذلك قد يؤدي إضافة حمض الهيوميك فى علف الدجاج البياض إلى التبكير فى النضج الجنسى.
 - ٣ - لم يؤثر إضافة حمض الهيوميك معنوياً على معامل شكل البيضة و الوزن النسبى للبياض و وحدات هير و كذلك معامل الصفار و هذه الصفات لم تتأثر معنوياً بعمر الدجاج. بينما ارتفع معنوياً الوزن النسبى للقشرة و سمك القشرة مقارنة بمجموعة الكنترول. فى حين ازداد سمك القشرة معنوياً عند عمر 32 أو 38 أو 44 أسبوع.
 - ٤ - أدى إضافة حمض الهيوميك خاصة المستوى العالى فى علف الدجاج البياض إلى زيادة معنوية فى عدد كرات الدم الحمراء و البيضاء و الهيموجلوبين و تركيز الكالسيوم و البروتين الكلى فى بلازما الدم مقارنة بالمجموعات الأخرى . وكان أعلى قيم تم الحصول عليها لكل من عدد كرات الدم البيضاء و الهيموجلوبين و البروتين الكلى عند عمر 44 أسبوع. بينما لم يتأثر معنوياً كل من مستوى الألبومين و أنزيمات GOT و الـ GPT و هرمون T_3 فى بلازما الدم بأضافة حمض الهيوميك أو بعمر الدجاج البياض.
 - ٥ - أدى إضافة حمض الهيوميك فى علف الدجاج البياض إلى زيادة معنوية للوزن النسبى للطحال و المبيض و كذلك طول قناة البيض خاصة مع المستوى العالى من حمض الهيوميك. بينما لم يتأثر معنوياً الوزن النسبى للذبيحة و الكبد و القونصة و القلب و قناة البيض مقارنة بمجموعة الكنترول.
- و بصفة عامة وجد أن إضافة حمض الهيوميك إلى غذاء الدجاج البياض أدى إلى تحسين إنتاج البيض و جودة القشرة و بعض الصفات الفسيولوجية و المناعية و خاصة المستوى العالى من حمض الهيوميك (200 ملجم / كجم علف).