

Study on the Effect of Humic Acid on Growth Performance, Immunological, Some Blood Parameters and Control Intestinal Closterdium in Broiler Chickens

Hala Salah*, El Sayed Masour, Reham RR** and Eman S Abd El Hamid*

(Bacteriology, Biochemistry* and Clinical Pathology Department**)

Animal Health Research Institute (Zagazig Branch)

ABSTRACT

Sixty broiler chickens of one day old were used to evaluate the effects of humic acid on body performance, leukogram and some biochemical parameters as well as effect in clostridium preferences (*C. perferenges*). At 20 of age 30 chicks were experementally infected with *C. perferenges*. Boiler chickens were divided into 4 equal groups (15 chicks each), 1st group healthy broiler chickens kept as control group, 2nd group healthy broiler chickens supplemented with 20 mg/kgm Bwt humic acid in drinking water for 5 successive days, 3rd group broiler chickens experimentally infected with *C. perferenges* non treated and 4th group broiler chickens experimentally infected with *C. perferenges* supplemented with 20 mg/kgm Bwt humic acid in drinking water for 5 successive day. Five chicks from each group were sacrificed at 1st and 10th day post supplementation. Two blood samples were taken for hematological biochemical study.

Broiler chicken infected with *C. perferenges* showed clinical signs as loss of appetite, drooping wings, ruffled feathers, depression, emaciation, diarrhea, mortality rate 33.33 %, significant decreases in weight gain, heterophile, phagocytosis, phagocytic index, Killing %, total protein, albumin, A/G ratio beside insignificant eosinophilia and basophilia coupled with significant elevation in feed conversion rate, leukocytic count, lymphocyte, monocyte, total, γ and α globulins, aspartate aminotransferase, alanine aminotransferase, Alkaline phosphates, uric acid and creatinine.

Healthy broiler chickens supplemented with humic acid showed significant increases in weight gains, leukocytic count, lymphocyte, phagocytosis, phagocytic index, killing %, total proteins, α , β and γ globulin, coupled with significant decreases in concentration of *C. perfringens* in intestinal content, feed conversion rate, heterophils, monocyte, aspartate aminotransferase, alanine aminotransferase, Alkaline phosphates, A/G ratio beside insignificant decrease of serum total protein, albumin, uric acid, creatinine and insignificant in eosinophilia and basophilia

Diseased broiler chicken supplemented with humic acid showed disappear clinical signs and improved body performance as well as hemato-biochemical parameters.

It could be concluded that humic acid induce reduction in concentration of *C. perfringens* in intestine beside improvement in weight gain, feed conversion rate, biochemical parameters and stimulate the immunity of broiler chickens.

INTRODUCTION

Antibiotics have been used in sub-therapeutic levels in chickens feed to inhibit the growth of intestinal pathogens and increase

body performance but created antibiotic residue and development of antibiotic-resistant bacteria (1). Antibiotics in feed enhance growth (2) researchers have looked for new

feed additives not harmful. Humic acid has potential to be an alternative to antibiotic growth promoters in broiler ration (3).

Humic acids are a class of compounds resulting from decomposition of organic matter is natural constituents of drinking water and soil (4). Using humic acid as feed additives in animal nutrition is new and used as a part of replacement therapy for digestive system disturbances as malnutrition and diarrhea (5), improve feed conversion rate (6) in broiler and (7) in turkey. Humic acid improved animal performance (8).

This study was conducted to determine the effects of humic acid on chicken performance, some immuno-logical and biochemical parameters as well as effect in growth intestinal *C. perferenges* in boiler chickens.

MATERIAL AND METHODS

Chickens

A total of 60, one-day old Hubbard mixed breed chicks were used in this trial, kept in wire floor batteries feed in startar ration obtained from Cairo Poultry Comp

Closterdium infection

At day 20 of age chicks were administered three oral inoculation of 2ml freshly prepared 24h *C. perferingens* type C. incubated thioglycolate broth culture of *C. perfringens* type C (1.5×10^9 organisms /ml) on alternate days (9).

Experimental design

At day 20 of age chicks were divided into 4 equal groups (15 chicks each), 1st group healthy broiler chickens kept as control group, 2nd group healthy broiler chickens supplemented with 20 mg/kgm Bwt humic acid in drinking water for 5 successive day, 3rd group broiler chickens experimentally infected with *C. perferenges* non treated and 4th group broiler chickens experimentally infected with *C. perferenges* supplemented with 20 mg/kgm

Bwt humic acid in drinking water for 5 successive day. treatment started in all groups at age of 25day.

Sampling

At 1st and 10th day post treatment two blood samples from each bird were taken 1st sample was taken on tube contain EDTA for determination leukogram (10), 2nd sample was taken for obtain clear serum for estimation T. protein (11) protein fraction (12) liver enzymes (aspartate aminotransferase, alanine aminotransferase) (13), Alkaline phosphates (14) uric acid (15), creatinine (16), Phagocytic activity, phagocytic index and Killing % were determined according (17).

Clostridium perfringens counting

At 1st day post infection before treatment, 1st and 10th day post humic acid supplementation intestine was dissected and one gram of intestinal content from each chicks was taken in sterile bags, then serial dilutions of each sample were made with normal saline and enumerated *C. perfringens* by pour plate method (18).

Body weight

All chickens were marked and individually weighed at start of the experiment and at 1st days post treatment, the amount of feed used was calculated. Where body weight gain, feed conversion rate were recorded

Statistical analysis

The obtained data were analysed (19).

RESULTS AND DISCUSSION

Chickens infected with *C. perferenges* showed loss of appetite, drooping wings, ruffled feathers, depression, emaciation, diarrhea, mortality rate 33.33% and increase concentration of *C. perfringens* number in intestinal content but using humic acid led disappear clinical signs, reduced mortality to 6.67% and *C. perfringens* number in intestinal content. Typical clinical signs were recorded (20) in broiler chickens infected with *C.*

perfringens. Same results was recorded (21) stated that humates inhibit many types of bacteria as *C. perfringens* is colonize in gastrointestinal tract of chickens. This finding confirms the result of (21) in broiler fed humates. Reduction in *C. perfringens* may be due to influence humic acid in proteins metabolism and carbohydrates in microbes lead to direct devastation of bacterial cells (23).

Humic acid induce significant increase in weight gains and improved in feed conversion rate but infected broiler chickens infected with *C. perfringens* revealed a significant decrease in weight gain and increase in feed conversion rate. Our results are parallel with (24) in healthy broiler chickens supplemented with humates. Also, humates resulted in a significant increase in weight gain (25) in quail. This finding may be due to humic acid can form a protective film on the mucus epithelium of the gastrointestinal tract against infections and toxins, thus improved utilization of nutrients in animal feed (26), increase feed consumption of bird (27). In addition, (28) found that *C. perfringens* infection induce significant decrease in weight gain and increase feed conversion rate in broiler chickens. Reduction in chickens weight infected with *C. perfringens* may be due to deleterious effect of the microorganism and retarded its metabolic activity and decrease absorption of nutrients from the inflamed intestine and diarrhea (29)

In the present study, broiler chickens supplemented with humic acid show significant increase in total leukocytic count, lymphocyte, phagocytosis, phagocytic index and killing % coupled with significant decrease in heterophils and monocyte, while chicks infected with *C. perfringens* show significant leukocytosis, lymphocytosis, monocytosis, beside significant decrease in phagocytosis, phagocytic index, Killing %, coupled with insignificant eosinophilia and basophilia. Our results are in complete harmony with those reported in broiler chicken received humic acid (30). Humic acid induce significant leukocytosis, lymphocytosis and heteropenia (31) in broiler chickens and (32) in quail

supplemented with humic acid. Humic acid induce leukocytosis due to increase of lymphocyte (33). Close similarity was seen between the finding and those obtained (34) mentioned that humic acid induce increase in phagocytosis and killing%. Elevation in phagocytosis, killing % may be due to immunostimulatory effects of humic acid (35). Same change in leukogram was recorded in duckling infected with *C. perfringens* (36). Birds respond to bacterial infection with a leukocytosis (37). Lymphocytosis and monocytosis may be associated with inflammatory disease (38).

Our findings revealed humic acid elicited insignificant decrease in total protein, albumin and significant increase in total, α , β and γ globulin in broiler chickens, but *C. perfringens* infection induce significant decrease in total protein, albumin and A/G ratio beside significant increase in total, γ , β and α globulins. Same change in protein picture was recorded (39-40) in chickens supplemented with humate. Increase in total, α , β and γ globulin in rats supplemented with humate (41). Humates have inhibiting effect on coliforms bacteria, secrete inflammatory agents increasing the protein synthesis in liver, and so decrease in serum total proteins (42). Same results are recorded (36) who reported that *C.* infection induces significant decrease in protein, albumin and significant increase in globulin. Reduction in total protein in infected chicks may be due to inappetance and male absorption of nutrients from inflamed intestine (13).

Humic acid induce significant reduction in aspartate aminotransferase, alanine aminotransferase, Alkaline phosphates and insignificant decrease in uric acid and creatinine; meanwhile, infected chickens show significant increase in aspartate aminotransferase, alanine aminotransferase, Alkaline phosphates, uric acid and creatinine. Same results were recorded (25,43) in broiler and quail supplemented with humic acid respectively. Same results were recorded (44) stated that *C. perfringens* infection induces significant raise aspartate aminotransferase,

alanine aminotransferase, Alkaline phosphates, uric acid creatinine in chickens. Elevation liver enzymes, uric acid and creatinine in infected broiler with were recorded (45) and this increase in liver enzymes, uric acid and creatinine have been associated with liver and kidney damage due to closterdial toxin (46).

It could be concluded that humic acid induce reduction in concentration of *C. perfringens* in intestine beside improvement in weight gain, feed conversion rate, biochemical parameters and stimulate the immunity of broiler chickens.

Table 1. Effect of humic acid on *C. perfringens* counts of intestinal content (Log 10) and mortality rate.

Parameter	healthy broiler chickens		Diseased broiler chickens	
	Non treated	treated	Non treated	treated
Clostridium perfringens	00	00	5.37±0.82	6.05±0.84
Counts/gm intestinal content	1st day post infection before treatment	00	00	00
	1st day	00	00	00
	10th day	00	00	00
Mortality rate	00	00	5(33.33%)	1(6.67%)

Table 2. Effect of *C. perfringens* and humic acid on body weight (Bwt) (gm/ chick), weight gain (WG), feed consumption (gm/bird) (F.C.), feed conversion rate (F.C.R.) of broiler chickens (n=5).

Parameter	Initial Bwt	Bwt	WG	F.C.	FCR
Non infected non supplemented with humic acid treated chicks (Control)	685.06±3.12	1178.18±4.32	493.12±4.80	685.76	1.39
Healthy chicks supplemented with humic acid	692.31±4.23	1205.30±5.95	512.99±4.93*	699.05	1.36
Diseased chickens non supplemented humic acid	690.60±3.86	1107.83±4.60	417.23±5.88*	646.31	1.55
Diseased chicks supplemented with humic acid	691.59± 4.83	1159.48±4.22	467.89±6.89*	680.95	1.46

*Significant at $P \leq 0.05$

Initial Bwt= body weight at 20 day of age

Table 3. Effect of humic acid and *C. perfringens* on leukogram, phagocytosis and Killing % of chicks (n=5).

Parameter	Control	Healthy humic acid treated	diseased			
			Non treated	humic acid treated		
				1 st day	10 th day	
leukocytic count	13.17±0.47	14.56±0.33*	14.99±0.19**	14.16±0.30	13.30±0.21	
Differential count	Lymphocyte	4.63±0.35	5.64±0.28*	5.80±0.23*	5.45±0.10*	4.75±0.34
	Heterophil	3.74±0.24	2.96±0.23*	2.84±0.19*	2.89±0.16*	3.55±0.23
	Eosinophil	1.51±0.30	2.34±0.43	2.07±0.39	1.72±0.32	1.63±0.28
	Basophil	1.75±0.25	2.48±0.39	2.14±0.42	1.90±0.38	1.88±0.21
	Monocyte	1.54±0.11	1.14±0.10*	2.49±0.29*	2.20±0.2	1.49±0.16
phagocytosis	58.32±1.69	66.05±1.84*	52.10±1.87*	56.43±1.46*	57.86±1.5	
Phagocytic index	2.14±0.24	3.42±0.32*	1.21±0.26*	1.98±0.18	2.07±0.18	
Killing %	42.18±1.28	46.50±1.40*	38.17±1.19*	41.34±1.21	42.03±1.94	

*Significant at $P \leq 0.05$

Table 4. Effect of humic acid and *C. perferinges* on protein profile of chickens (n=5).

Parameter	Control	Healthy humic acid treated	Diseased			
			Non treated	humic acid treated		
				1 st day	10 th day	
T. protein(gm/dl)	5.06±0.13	4.84±0.27	4.62±0.12*	4.47±0.21*	4.85±0.32	
Albumin(gm/dl)	2.69±0.19	2.13±0.31	1.86±0.21*	2.02±0.26	2.46±0.28	
Globulin (gm/dl)	α	1.08±0.03*	1.10±0.04*	1.02±0.06	0.99±0.08	
				β	0.64±0.03*	0.58±0.04
					γ	1.01±0.06
Total	2.37±0.11	2.71±0.10*	2.76±0.12*	2.61±0.17	2.39±0.24	
A/G Ratio	1.44±0.21	0.79±0.19*	0.67±0.17*	0.86±0.20	0.98±0.43	

*Significant at P ≤ 0.05

** Significant at P ≤ 0.01

Table 5. Effect of humic acid and *C. perferinges* on liver and kidney function of chickens (n=5)

Parameter	Control	Healthy humic acid treated	Diseased		
			Non treated	humic acid treated	
				1 st day	10 th day
AST(μ/L)	12.30±0.62	10.48±0.41*	15.07±0.69*	13.90±0.31*	11.74±0.93
ALT(μ/L)	32.16±0.84	29.04±0.78*	36.28±0.61**	34.53±0.38*	31.89±0.868
ALP.(I.U/ml)	19.41±0.52	16.83±0.69*	22.16±0.79*	20.10±0.59	19.03±0.69
uric acid(mg/dl)	4.74±0.29	4.20±0.31	6.14±0.28*	5.49±0.14*	4.12±0.43
Creatin. (mg/dl)	1.06±0.09	0.94±0.07	1.72±0.21*	1.30±0.10	0.99±0.15

*Significant at P ≤ 0.05

** Significant at P ≤ 0.01

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الملخص العربي

دراسة تأثير حمض الهيومك على معدل النمو ، بعض مكونات الدم والتحكم في ميكروب الكلوسترديم في أمعاء كتاكيت التسمين

هاله صلاح*، السيد منصور ، ريهام رضا الرشيدى** ، ايمان سعودى عبد الحميد*
معهد بحوث صحة الحيوان (فرع الزقازيق- اقسام البكتريولوجى الكيمياء* والباثولوجيا الاكلينيكية**)

تم إجراء هذا البحث على ٦٠ كتكوت عمر يوم واحد تم تقسيمهم إلى ٤ مجموعات متساوية (١٥ كتكوت فى المجموعة). المجموعات الأولى والثانية منهم بحاله صحيه جيده والمجموعات الثالثه والرابعه يتم إصابتهم إصابة اصطناعيه بالكلوسترديم بيرفرنجيز نوع C بجرعه (١,٥سمx١٠ ٩) عن طريق الفم. المجموعة الأولى كتاكيت سليمه ظاهريا واكلينكيا ولم يتم امدادها بحمض الهيومك (مجموعة ضابطة)، المجموعة الثانية كتاكيت سليمه ظاهريا واكلينكيا ويتم امدادها بحمض الهيومك (٢٠ مجم/كجم من وزن الجسم) فى مياة الشرب لمدة ٥ أيام. المجموعة الثالثه كتاكيت مصابة بالكلوسترديم بيرفرنجيز ولم تعالج باى أدوية اما المجموعة الرابعه كتاكيت مصاب بالكلوسترديم بيرفرنجيز وتعالج باستخدام حمض الهيومك (٢٠ مجم/كجم من وزن الجسم) فى مياة الشرب لمدة خمسة أيام متتالية. يتم دراسته كفاءه حمض الهيومك فى علاج عدوى الكلوسترديم بيرفرنجيز وذلك عن طريق اعاده عزل الميكروب وتسجيل عدد الوفيات ومدى اختفاء الاعراض الظاهريه للمرض وتأثير المرض والعلاج على معدل النمو. يتم ذبح عدد ٥ كتكوت عند اليوم الاول والعاشر من نهايه العلاج ويتم اخذ عينتين دم من كل طائر لقياس بعض الوظائف المناعيه والبيوكيميائيه.

أوضحت الدراسة أن حمض الهيومك ادى إلى حدوث زيادة معنوية فى وزن الجسم المكتسب، استهلاك العلف، العدد الكلى لكرات الدم البيضاء، قوة اللاتهام، نسبة القتل، الجلوبيولين الكلى ، α, β, γ ، جلوبيولين. كما لوحظ ان استخدام حمض الهيومك ادى الى تقليل عدد ميكروب الكلوسترديم فى العينات المفحوصه، معدل التحويل الغذائى، خلايا الهييتروفيل، خلايا الملتهمه الكبيره، النسبه بين الزلال والجلوبيولين، انزيمات الكبد (ALP- ALT-AST) بجانب وجود نقص معنوى فى البروتين الكلى، الزلال، حمض اليوريك، الكرياتينين ونقص غير معنوى فى الخلايا الحامضيه والقاعديه.

وتشير النتائج ان الاصابه بالكلوسترديم بيرفرنجيس فى كتاكيت التسمين ادت الى ظهور اعراض تتمثل فى قله الاكل، نكش الريش الاسهال والضعف مع نسبة عالية من الوفيات (٣٣,٣٣%). وجود نقص معنوى فى وزن الجسم المكتسب وزيادة معنويه فى معدل التحويل الغذائى، العدد الكلى لكرات الدم البيضاء، الخلايا الليمفاويه، الخلايا الملتهمه الكبيره، الجلوبيولين الكلى ، α, β, γ ، انزيمات الكبد (AST, ALT, ALP) حمض اليوريك والكرياتينين بجانب وجود نقص معنوى فى خلايا الهييتروفيل، وزن الجسم المكتسب، خلايا الهييتروفيل، قوة اللاتهام ونسبه القتل، البروتين الكلى ، الزلال، النسبه بين الزلال والجلوبيولين مصحوبه بزياده غير معنويه فى الخلايا الحامضيه، الخلايا القاعديه.

من كل ما سبق يمكن القول ان حمض الهيومك له تأثير فعال على ميكروب الكلوسترديم بيرفرنجيس وادى الى تحسن ملحوظ فى وزن الجسم ومعدل التحويل الغذائى وصورة الدم البيضاء والوظائف البيوكيميائيه.