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BIOLOGICAL IMMUNE STIMULANTS EFFECTS ON IMMUNE RESPONSE, BEHAVIOURAL AND PRODUCTIVE PERFORMANCE OF BROILERS.

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ABSTRACT: This study was conducted to evaluate the effect of different biological immune stimulants; humic acid, β glucan and humic acid, β glucan mixture; on physiology, performance, Behaviour, and immune response to NDV and IB. A total of 540 broiler cobb chicks, were used in this study, housed in experimental unit at Faculty of Veterinary Medicine, Cairo University, Egypt, with stocking density 10/ m², using 12 pens, 45 chicks for each . Feed and water were provided *ad libitum* via trough feeders and bell drinker. Birds were divided into four equal groups. T1, T2, T3, and T4; each of them 135 chicks, three replicates, (45 each). T1 control group didn't receive any supplement, T2 group receive humax® (consists of 200 mg humate /ml) in drinking water, T3 group took meduline® (consists of 100mg β glucan /ml), T4 receive blue Immune® (consists of 100 mg humate and 50 mg β glucan /ml); all given day after day 0.5 cm/liter drinking water. Data collected were, productive performance (final body weight, feed intake, feed conversion ratio, mortality rate and European performance index, behavioral measurements; (feeding behaviour, drinking behaviour, leg and wing stretching, preening, leg scratch and resting behaviour), oxidative stress parameter and antibodies titer for NDV and IB was estimated. Results revealed significance different between different immune stimulant as humic acid either alone or in combination with β glucan recorded lower feed intake, lower feed conversion ratio, higher feeding percentage, higher drinking and welfare indices, in the same time immune responses toward NDV was the same in T2 and T4 while antibodies titer against IBV did not differed. Conclusively, it was obvious that with humic acid alone or plus β glucan has a positive effect on physiology, performance, behaviour and immune response of broiler.

Key Words: Immunostimulant, Behaviour, Oxidative stress, Humate, β glucan

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INTRODUCTION

In recent years there is an increase in bacterial resistance to antibiotics in both human and animal population, as well as an increasing consumer concern for animal drug residues in meat (Donaghue 2003). Due to the ban of most antibiotics growth promoters in the world, finding alternatives means of boosting disease resistance without the antibiotics is considerable practical interest of animal production. In this issue, the use of immune modulators for increasing host defense and immunocompetence is receiving more attention.

Numerous antibiotics alternatives are in use or have been proposed as a mean to eliminate pathogens or to improve growth and feed conversion, including probiotics, enzymes, bacteriocins, antimicrobial peptides and bacteriophages (Joerger 2003) as well as, herbal compounds and organic acids. These substances exert their effects by influencing gastrointestinal flora and digestion process. Among these alternatives, humic acid and β glucan. Using humic acid in animal nutrition has a very short history. Lenk and Benda (1989) and Griban et al. (1991) first used humic acid to improve the immune system of calves. Ku- hnert et al. (1991) used humic acid to treat digestive disorders and diarrhea in dogs and cats.

Humic acid has been studied and has been reported to have positive impact on the growth performance of birds. Humic acid inhibits bacterial and fungal growth, thus decreases levels of mycotoxins in feed (Humin Tech, 2004). Also, it has been used as an antidiarrheal, analgesic immunostimulant and antimicrobial agent in veterinary practice in Europe (EMEA 1999).

Beta-glucans (β glucan) are members of a group of physiologically active compounds generally represent highly conserved structural components of cell walls in yeast, fungi, seaweed and plants. Glucan's role as a biologically

active immunomodulator has been well documented for over 60 years. Biological effects of glucans include stimulation of immunity, activation of bone marrow cell production, anti-cancer effects, lowering of blood cholesterol and amelioration of stress (Vetveicka et al., 2007).

Therefore the goal of this study was to detect the effect of either humic acid alone or in combination with β glucan on the performance and behavior of broilers .Also, immunological effects and physiological profile were investigated.

MATERIALS AND METHODS

Birds and Housing: A total of 540 broiler Cobb chicks, were used in this study, housed in experimental unit at Faculty of Veterinary Medicine, Cairo University, Egypt, with stocking density 10/ m², using 12 pens (45 chicks /pen). Feed and water were provided *ad libitum* via trough feeders and bell drinker. The birds were vaccinated against NDV, and IB. Diets used were in pellets form and formulated to meet the nutrient requirement of the broiler chicks (Table1), according to recommendations of the national research council NRC (1994).

Experimental design: Birds were divided into four groups. T1, T2, T3, and T4; each of them was 135 chicks, representing three replicates, (45 each). T1 group, were birds that received plain water throughout the study. T2 group, birds were supplemented with Humax® (consists of humates 200mg /ml), dosage 0.5 ml/liter drinking water every other day. T3 group: birds were supplemented with Meduline® (consists of 100mg β glucan /ml), they were administered in drinking water, 0.5 cm/liter every other day. T4 group: birds were received Blue-Immune® (consists of humate 100mg in combination with 50 mg β glucan /ml), birds received 0.5cm /liter drinking water every other day.

Measurements: The following parameters were recorded:

1- Productive performance: final body weight, feed intake, feed conversion ratio

as well as mortality rate were recorded. Also, European efficiency index was calculated (Mahmoud et al., 2009).

2-Immunological parameters: humoral immune response against Newcastle disease and infectious bronchitis was detected. Fifteen serum samples from each group were taken at 14, 21, 28 and 35 days of age to determine humoral immune response against Newcastle disease, using the standard haemagglutination inhibition (HI) test according to Allan and Gough (1974). Also, 15 serum samples from each group at 35 days of age were collected to evaluate humoral immune response against IBV, using commercial ELISA system according to Case et al. (1982).

3-Behavioral measurements: the following behavioral patterns were observed and measured; feeding and drinking behavior as well as welfare index behaviour (leg and wing stretching, preening, leg scratch and resting behavior) and eliminative behaviour (Table 2). Program of measuring were applied according to (Altman 1974); through daily instantaneous sampling for 10 minutes twice daily/pen. Observation session was divided into short intervals and recorded whether the behaviour pattern was occurred; the results were expressed as the percentage of birds performing the behaviour (Reiter and Bessei, 2000).

4-Blood biochemical measurements: blood samples were collected at 21th day and 35th day, from wing vein in heparinized test tubes. The following parameters were measured:

- a) Glutathione activity (GSH) was estimated spectrophotometrically using heparinized whole blood and the method based on the reduction of 5,5` dithiobis (2- nitrobenzoic acid) (DTNB) with glutathione (GSH) according to Beutler et al. (1963).
- b) Lipid peroxide (Malondialdehyde) was estimated spectrophotometrically using heparinized plasma and the method

based on Thiobarbituric acid (TBA) reacted with malondialdehyde (MDA) in acidic medium at temperature of 95°C for 30 min according to Ohkawa et al. (1979).

- c) Catalase activity was estimated using also heparinized plasma and the method based on catalase reacted with a known quantity of H₂O₂, according to Aebi (1984).
- d) Total antioxidant capacity was measured using the reaction of antioxidants in the sample with a defined amount of exogenously provide hydrogen peroxide according to Koracevic and Koracevic (2001).

Statistical analysis: the data were statistically analyzed using SAS Software. The comparison of means was done with Duncan, 1955, in order to get regression equation and correlation coefficients. The level of statistical significance was pre-set at $p \leq 0.05$.

RESULTS AND DISCUSSION

According to the results of this experiment, humic acid and mixture of humic acid and β glucan added as a supplement in the water, had significant effect on the performance of broilers, final body weight, feed conversion rate and European coefficient index. The average final body weight, total feed intake and feed conversion values of treated groups were recorded in table (3). Supplementation of humic acid plus β glucan (T4) resulted in a significant increase in the final body weight 2107 ± 25.9 g than that of humic acid, β glucan and control group ($P \leq 0.05$). Also, T₄ group (Humic acid plus β glucan) showed significant increase in total feed intake (3426 ± 30 g) compared with the other groups ($P \leq 0.05$).

Concerning feed conversion ratio, the humic acid group (T2) indicated a significance decrease 1.56 ± 0.01 than the other groups. In addition, mortality rates of broilers were determined in the present study. The results revealed no mortalities

for the control group (T1) and humic acid group (T2) at ($P \leq 0.05$) compared with β glucan group and humic acid plus β glucan groups. Confirming previous findings, that humic acid and esterified glucomannan can be used as a growth promoters in broiler diets and they can improve the gut health too (Seyed et al., (2012) and Kocabagli et al., (2002). Moreover, Wang et al., (2008) reported that Humic substances have been found to improve growth performance, relative lymphocyte count and meat quality.

Feed intake was affected by inclusions of humic acid or combination of both humic acid and β glucan throughout the study. Birds supplemented with humic acid had lower level of feed intake as compared to other experimental groups and best feed conversion ratio was observed moreover high European coefficient index. These findings were in accordance with the reports of Kocabagli et.al., (2002) and Celick et.al., (2014). It is assumed that due to proteins, vitamins, digestive enzyme and many other immune stimulating agent and antibacterial substance in humic acid, have a significant role in the health and productivity of birds. The study recorded that combination of humic acid and β glucan recorded a stronger effect on the performance of broilers (final body weight) as reported earlier by Santin et .al., (2001), humic acid and esterified glucomannan had a significant effect on crypt depth of villiae of the jejunum of treated birds compared to control group. It is obviously known that, growth of villiae will be depending on pH, micro flora and toxic substances in the intestine. Though, humic acid will have the ability in reducing pH and concentration of harmful bacteria in intestine. Confirm the evidence of (Shermer et .al., (1998) that humic acid could have positive effect on animal performances via digestive tract ecosystems.

According to Rath, et. al., (2006) that humic acid have the ability to create protective layers over the epithelial

mucosal membrane of digestive tract against the penetration of toxic and other bacterial contaminated substances, moreover recorded that it acts as detoxification agent in intestine and also increases immune receptors in digestive tract over preserving the beneficial pathogens. Also Humin Tech, (2004) reported that humic acid stabilize the intestinal flora and thus ensure an improved utilization of nutrients by the host animal. This leads to an increase in live weight of the animal without increasing the amount of feed given. Confirmatory results derived from other study for Kocabagli et. al., (2002) found that humic acid in feed improved growth of broiler by increasing digestion of protein and trace element utilization.

Glucomannan also secretes more enzymes in projected villiae, which finally will enhance the absorption and digestion of food particles. Moreover, glucomannan will have effect to create protective layers on intestinal mucus to inhibit penetrations of toxic substances and harmful bacteria in the gut (Hampson, 1986). At the same time; (Humin Tech 2004) recorded that replacing antibiotics with humic acid as growth promoter in animal feed excludes the possibility of antibiotic residues or microbial resistance Simultaneously, as a result of a better feed conversion rate and enhanced absorption, nitrogenous wastes and nitrogen odor are reduced.

This finding was in accordance with the Kocabagli et. al., (2002), and Celick et. al., (2008), who found that feed conversion ratio was improved by inclusions of both humic acid and esterified glucomannan.

Regarding immune status of the birds supplemented by humic acid or humic acid plus β glucan the data revealed that there was no change between different groups in the antibodies titer against NDV until 28 days but later on, at the age of 35 days birds supplemented with humic acid alone or in combination with β glucan achieved higher antibodies titer than the other

groups. Klocking, (1994) and EMEA, (1999); reported that humic acid has immunostimulant, anti-inflammatory and antiviral effects. The effects of humic acid on antibody titers are due to its antiviral properties (Enviromate, 2002), phagocytic activity of leukocytes, activation of neutrophils (Chang-Hua et al., 2003), improving nutritive value of feed (Kocubagli et al., 2002), ability to block colonization of pathogens in the gastrointestinal tract (Klocking, 1994) and improving immune functions (Eren et al., 2000).

Welfare encompasses both the physical and psychological well-being of animals. Behavior is identified as important for the wellbeing of chickens, including feeding, drinking, perching, scratching, foraging, and comfort and resting behavior (Shields, 2004 and Webster, 2005).

No studies appear to have tested the possible effect of humic substance or beta glucan on behavioral performance of broilers. Furthermore, the involvement of various arrays of measurement to properly evaluate feeding, drinking, comfort and resting behavior in broilers is not well implemented. Interestingly, the present results declared that humate supplementation significantly increased feeding and drinking behavior in broilers (table (5) $P \leq 0.05$). Moreover, combination of both humate and β glucan increased feeding activity than the control and β glucan group. These data are in accordance with previously mentioned results, as humate or combination of both humate and glucan increased performance of broilers in the form of high level of weight gain and feed conversion rate as well as decrease mortality rate.

Regarding body care and comfort behavior of broilers, many studies have shown that comfort behavior important for body maintenance and care of feathers, such as wing and leg stretching, wing flapping, body shaking and preening (Duncan, 1981 and Nicol, 1987). Preening

keep chicken's feathers and skin in healthy condition and also balance lipid levels in the feathers (Shields, 2004). Results recorded in table (5) showed marked increase in wing – leg stretching, body scratch and preening in broilers supplemented with humic acid ($P \leq 0.05$). While birds supplemented with humic acid plus β glucan, also showed increase in leg and wing stretch and preening activity. While the resting behavior of the birds treated with humate or combined treatment of humate and β glucan revealed significant decrease in resting behavior. Humic acid treated birds displayed significantly higher ($p \leq 0.05$) elimination than the control and other treated groups and this may be attributed to increasing the amount of water intake.

The present results regarding performance (final body weight, feed conversion and mortality rate) and immunological parameters as well as behavioral activity were paralleled with the physiological changes. The antioxidant measurement (table 6) recorded that supplementation of humic acid separately or in combination with β glucan for broilers has a powerful antioxidant activity and could protect the cells from oxidative stress and damage by increasing the glutathione reductase, total antioxidant and catalase activity and therefor change the toxic free radicals to another less harmful molecule, and also decrease the malondialdehyde lipid peroxidase, so protect the cells from lipid peroxidation and production of the toxic free radicals.

The obtained data and antioxidant activity go hand by hand with the previous studies which reported that humic substances can modulate the toxicity of pollutants, and the bioavailability of metals (Paquin et al., 2002; Glover and Wood, 2004) and can alter pH, ionic concentration and enzymatic activity (Timofeyev et al., 2006). As Vaškova et al., (2011) who pointed out that the electrophilic properties of humic substance markedly balance the

mitochondria redox status. Moreover, strong scavenging properties against hydroxyl radicals and to a lesser extent against superoxide radicals were found. In accordance with our results Kofuji. et. al., (2012) revealed that the antioxidant activity of β glucan was significantly higher than that of various polymers that are used as food additives. The ability of β glucan to scavenge ROS is a precious property for the prevention of various diseases and contributes to enhancing health.

CONCLUSION

To summarize our data, our report suggests that humic acid are biologically active immunomodulators affecting both the humoral and cellular branches of immune reactions as well as achieve high productive performance and good welfare index among broilers. In addition, both humic substance and β glucan are working in synergy to enhance growth and performance of broilers.

Immunostimulant, Behaviour, Oxidative stress, Humate, and β glucan.

Table (1): Formula and chemical composition of basal diets .

Ingredients	Starter	Grower	Finisher
Yellow corn	61.4	68.3	73.4
Soya bean meal (46%)	29.9	20.3	15.5
Full fat soy (35%)	1	1	3.2
Corn gluten meal (62%)	3.5	6.1	3.7
DL-Methionine	0.26	0.27	0.3
L-Lysine HCL	0.265	0.450	0.470
L-Threonine	0.085	0.085	0.3
Mono calcium phosphate	1.17	1.13	0.75
Lime stone	1.63	1.575	1.5
Salt	0.24	0.24	0.23
Sodium bicarbonate	0.2	0.2	0.3
Choline chloride (60 %)	0.05	0.05	0.05
Premix	0.3	0.3	0.3
Total	100	100	100
Calculated chemical composition (%)			
Crude protein (%)	21.6	19.5	17.07
Metabolizable energy (Kcal/kg)	3050	3150	3200
Calcium (%)	0.1	0.95	0.9
Phosphorus (total) (%)	0.64	0.6	0.57
Available phosphorus (%)	0.5	0.48	0.46
Methionine %	0.63	0.63	0.61
Lysine %	1.3	1.21	1.12
Threonine %	0.95	0.85	0.77
Sodium %	0.17	0.17	0.17

Each 3 Kg of premix contains: Vitamins: A: 12000000 IU; Vit. D3 2000000 IU; E: 10000 mg; K3: 2000 mg; B1:1000 mg; B2: 5000 mg; B6:1500 mg; B12: 10 mg; Biotin: 50 mg; Choline chloride: 250000 mg; Pantothenic acid: 10000 mg; Nicotinic acid: 30000 mg; Folic acid: 1000 mg; Minerals: Mn: 60000 mg; Zn: 50000 mg; Fe: 30000 mg; Cu: 10000 mg; I: 1000 mg; Se: 100 mg and Co: 100 mg

Table (2): Description of each behaviour pattern measured during experiment

Behavioural patterns	Description
Feeding	Head extended towards available feed resources while beak in or above the feeder and appear to be ingesting feed.
Drinking	Beak in contact with water in or above the drinker and appear to be drinking water.
Wing/leg stretching	Extending one wing and one leg at the same side of the body.
Preening	Beak related behaviour that beak touches the plumage of the bird itself.
Body Scratching	Scratching parts of the body especially head and neck with feet.
Resting	Head rested on objects (Litter or another bird) while sitting
Eliminative Behaviour.	Behaviour associated with elimination of droplet from the body.

Table(3): Productive performance in different treated groups

	T1	T2	T3	T4
Total feed intake (g)	3372 ± 23.3 ^b	3238 ± 41.3 ^d	3258 ± 43.3 ^c	3426±3.3 ^a
Final body weight (g)	2037 ± 25.98 ^c	2063 ± 25.8 ^b	1951± 26 ^d	2107± 25.9 ^a
Food conversion rate	1.65 ± 0.00 ^b	1.56 ± 0.01 ^d	1.66 ± 0.00 ^a	1.62± 0.00 ^c
Mortality rate (%)	0 ± 0.00 ^c	0 ± 0.0 ^c	4.54 ± 0.28 ^a	2.42± 0.17 ^b
European coefficient index	352.7 ^c	372.8 ^a	320 ^d	362.6 ^b

T1: received plain water, T2: supplemented with humic acid, T3: supplemented with β glucan, T4 supplemented with humic acid plus β glucan.

Note. Different subscripts within a row indicate a significant treatment effect ($p < 0.05$)

Table (4): Newcastle and infectious bronchitis diseases antibodies titer in different treated groups

	T1	T2	T3	T4
NDV14	5.6 ± 0.4 ^a	5.6 ± 0.24 ^a	6.00± 0.4 ^a	6.4± 0.24 ^a
NDV21	7.8 ± 0.2 ^a	8 ± 0.4 ^a	7.2 ± 0.2 ^a	5.8 ± 0.2 ^b
NDV28	7 ± 0.0 ^a	7 ± 0.0 ^a	7.00± 0.0 ^a	6.6 ± 0.24 ^a
NDV35	6.8 ± 0.2 ^{ab}	7.4 ± 0.24 ^a	6.2 ± 0.2 ^b	7.4± 0.40 ^a
IB35	1357± 499.2 ^a	1290.2± 318.96 ^a	1266.4± 208.36 ^a	1318.6 ± 685.12 ^a

T1: received plain water, T2: supplemented with humic acid, T3: supplemented with β glucan, T4 supplemented with humic acid plus β glucan.

Note. Different subscripts within a row indicate a significant treatment effect ($p < 0.05$)

Immunostimulant, Behaviour, Oxidative stress, Humate, and β glucan.

Table (5): Welfare index and behaviour in different treated groups measured as percentage

	T1	T2	T3	T4
Feeding %	19.97 \pm 4.61 ^d	28.06 \pm 4.1 ^a	23.7 \pm 2.6 ^c	25.5 \pm 4.6 ^b
Drinking %	31.26 \pm 4.1 ^b	32.4 \pm 2.6 ^a	31.95 \pm 4.6 ^c	26.65 \pm 4.1 ^d
Leg and wing stretch %	21.4 \pm 3.6 ^b	23.9 \pm 4.1 ^a	18.77 \pm 1.6 ^d	19.73 \pm 4.6 ^c
Body scratch %	12.1 \pm 3.1 ^b	14.37 \pm 1.6 ^a	10.36 \pm 4.6 ^c	10.28 \pm 4.5 ^d
Preening %	22.4 \pm 4.6 ^b	25.23 \pm 2.1 ^a	20.82 \pm 4.1 ^d	22.34 \pm 1.4 ^c
Resting %	83.74 \pm 3.16 ^a	79.42 \pm 4.18 ^c	81.29 \pm 2.4 ^b	78.02 \pm 4.6 ^d
Elimination %	7.65 \pm 0.57 ^d	12.9 \pm 0.6 ^a	9.36 \pm 0.75 ^b	8.7 \pm 0.57 ^c

T1: received plain water, T2: supplemented with humic acid, T3: supplemented with β glucan, T4 supplemented with humic acid plus β glucan.

Note. Different subscripts within a row indicate a significant treatment effect($p < 0.05$)

Table (6): Oxidative status in different treated groups.

	Day	T1	T2	T3	T4
Glutathione Reductase (U/ml)	21 th	59.14 \pm 0.6 ^b	67.72 \pm 1.41 ^a	59.01 \pm 0.53 ^b	60.07 \pm 0.22 ^b
	35 th	54.34 \pm 1.01 ^c	66.35 \pm 2.24 ^a	54.97 \pm 1.00 ^c	59.29 \pm 0.62 ^b
Malondialdehyde(U/ml)	21 th	7.09 \pm 0.38 ^a	4.64 \pm 0.16 ^b	5.56 \pm 0.42 ^b	5.00 \pm 0.63 ^b
	35 th	6.81 \pm 0.69 ^a	2.42 \pm 0.17 ^c	3.1 \pm 0.17 ^c	4.53 \pm 0.508 ^b
Catalase (U/ml)	21 th	288.7 \pm 3.16 ^b	326.6 \pm 15.33 ^a	336.7 \pm 16.5 ^a	325.19 \pm 24.5 ^a
	35 th	241.4 \pm 13.47 ^b	371.17 \pm 18.4 ^a	261.9 \pm 12 ^b	253.8 \pm 24.3 ^b
Total antioxidant (U/ml)	21 th	0.68 \pm 0.57 ^b	1.08 \pm 0.06 ^a	0.96 \pm 0.039 ^a	1.01 \pm 0.05 ^a
	35 th	0.52 \pm 0.01 ^d	1.17 \pm 0.068 ^a	0.67 \pm 0.031 ^c	0.86 \pm 0.03 ^b

T1: received plain water, T2: supplemented with humic acid, T3: supplemented with β glucan, T4 supplemented with humic acid plus β glucan.

Note. Different subscripts within a row indicate a significant treatment effect($p < 0.05$)

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الملخص العربي

تأثير المحفزات المناعية البيولوجية المختلفة علي الإستجابة المناعية والكفاءة الإنتاجية والسلوكية

في قطعان التسمين

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أجريت هذه الدراسة لتقييم بعض المنشطات المناعية البيولوجية المختلفة حمض الهيوميك، والبيتا جلوكان ومزيج من حمض الهيوميك والبيتاجلوكان معا) ودراسة تأثيرها على الأداء الإنتاجي والأنشطة السلوكية، وبعض المعايير الفسيولوجية والاستجابة المناعية لمرض النيوكاسل والتهاب القصبات المعدي في بداري التسمين. تم استخدام 540 طائر من الدجاج اللحم سلالة كوب، واجريت الدراسة في وحدة أبحاث الدواجن بقسم الصحة والرعاية البيطرية بكلية الطب البيطري جامعة القاهرة. تم تقسيم الطيور الي 4 مجموعات اساسية كل مجموعة 135 طائر ، المجموعة الضابطة وثلاثة مجموعات ؛ ثلاثة مكررات لكل مجموعة 45 طائر لكل مكرر. المجموعة الضابطة لا تتلقى أية إضافة في مياه الشرب؛ المجموعة الثانية تتناول الهيوميكس (يحتوي علي حمض الهيوميك 200 مجم / مللي) ؛ والمجموعة الثالثة تتناول المديولين (مكون من بيتا جلوكان 100 مجم/مللي)؛ والمجموعة الرابعة تتناول بلواميون (مكون من مزيج من الهيوميك 100مجم/مللي مع البيتا جلوكان 50مجم/مللي) ؛ جميع الإضافات تكون في مياه الشرب بمعدل 0.5 سم/لتر ماء الشرب يوم بعد يوم طوال فترة التربية؛ وتم قياس الأتى الوزن النهائي للطيور، متوسط استهلاك العلف، نسبة التحويل الغذائي، نسبة النفوق ومعامل كفاءة الأداء الأوروبي ، ومن الأنشطة السلوكية سلوك تناول الغذاء، والشرب ، سلوك الهندمة وتنظيف الريش وسلوك الراحة ؛ كما تم قياس مضادات التأكسدة وتقدير الأجسام المناعية ضد النيوكاسل والإلتهاب الشعبي المعدي. ، وأسفرت النتائج عن الآتى:-المنشط المناعي الذي يحتوي علي حمض الهيوميك سواء أكان ذلك منفردا أو ممزوجا مع البيتاجلوكان سجل اعلى اوزان واكل استهلاك العلف، أقل معامل تحويل غذائي، اما بنسبة لسلوك تناول الغذاء وشرب المياه فكان أعلى أيضا في مجموعة حمض الهيوميك منفردا او مجتمعا مع البيتا جلوكان، في الوقت نفسه الاستجابات المناعية للنيوكاسل كانت اعلي في المجموعات التي تتناول الخليط من الهيوميك والبيتا جلوكان و الهيوميك منفردا ثم البيتاجلوكان منفردا. ولذا خلص البحث الى الآتى: إستخدام حمض الهيوميك منفردا أو مع البيتا جلوكان له أثر إيجابي على معدلات الإنتاج والأنشطة السلوكية والإستجابة المناعية للدجاج اللحم.