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The Effect of Gibberellic Acid and Vitamin E Addition to the Drinking Water of Broiler Chickens on Some Productivity, Physiological and Immunological Parameters

تأثير أضافة حامض الجبرليك وفيتامين E لماء الشرب في بعض الصفات الانتاجية والفسلجية والمناعية لأفراخ دجاج اللحم

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Abstract

Ninety chicks one day old were divided into three treatments. In which 30 chicks: control treatment was considered the first one, 150 $_{ppm}$ Gibberellic acid were added to the drinking water of treatment two, and 150 $_{ppm}$ Gibberellic acid and 30 mg $_{per}$ liter vitamin E for the third treatment. Weekly, chick's weights were taken for the whole of studying period of 49 days. Liver weight, dressing percentage, and humoral immunity against Newcastle virus disease and liver enzymes ALT and AST were measured 2 times during the experiment. These 2 tests were done at 32 and 49 days old.

Results of the experiment had shown no significant differences in weekly body weight and dressing percentage. Meanwhile, average liver weights of T2 and T3 were significantly higher comparing with the control on the age of 49 days at ($p\leq0.05$). The antibody titers against NDV (both tests mean) were significantly higher ($p\leq0.05$) in T2 compared to the average of the two periods for the third and the first sets individually.

Aspartate aminotransferase (AST) effect was significantly higher in T2 and T3 comparing with the first set at 49 days aged broiler chickens, but 32 days old test showed the reverse. On the other hand, alanine aminotransferase (ALT) shows no significant differences between treatments in both times. Generally speaking, significant increases in the average levels of the three treatments of AST and ALT at the second test (49 days old chickens) as compared with average them at the first test.

Key words : Gibberellic Acid , Vitamin E , Antibodies , AST , ALT .

الخلاصة

لدراسة التأثيرات الايجابية والسلبية لحامض الجبرليك على دجاج اللحم تم تربية 90 فرخ دجاج بعمر يوم واحد قسمت الى ثلاث معاملات كل معاملة احتوت على 30 فرخًا وكانت المعاملات كالأتي :-

المعاملة الأولى:- أعطيت ماء شرب خالي من أي أضافة وعدُت كمعاملة سيطرة.

المعاملة الثانية :- أعطيت ماء شرب مضافًا اليه حامض الجبرليك بجرعة قدرها 150 جزء بالمليون .

المعاملة الثالثة :- أعطيت ماء شرب مضافًا اليه حامض الجبرليك بجرعة قدرها 150 جزء بالمليون و فيتامين E بجرعة قدرها 30 ملغم/ لتر ماء.

تم وزن الافراخ أسبوعيا طيله مدة الدراسة البالغة 49 يوم وعلى فترتين تم أحتساب وزن الكبد و نسبة التصافي والمناعة الخلطية ضد مرض نيوكاسل وأنزيمات الكبد Aspartate aminotransferase, Alanine aminotransferase.

بينت نتائج التجربة عدم وجود فروقًا معنوية بوزن الجسم الاسبوعي ونسبة التصافي بينما كان هناك ارتفاع معنوي على مستوى ($أ< 0.05$) لمعدل وزن الكبد للمجموعتين المعاملتين مقارنةً بمجموعة السيطرة بعمر 49 يوم ، وكان هناك أرتفاع معنوي في المناعة الخلطية ضد مرض نيوكاسل بمستوى ($أ< 0.05$) لمتوسط فترتي التجربة للمعاملة الثانية على متوسط فترتي التجربة للمعاملتين الثالثة والاولى ، اما بالنسبة لانزيمات الكبد فكان هناك تأثيرًا معنويًا للمعاملتين الثانية والثالثة على المعاملة الاولى لمستوى AST بعمر 49 يوم وخلاف ذلك التأثير كان في عمر 32 يوم بينما لم يكن هناك فروق معنوية بين المعاملات لكلا الفترتين لمستوى ALT وبصورة عامة كان هناك ارتفاع معنويًا لمتوسط مستويات المجاميع الثلاثة لأنزيمات الكبدAST و ALT بعمر 49 يوم على متوسط مستويات المجاميع الثلاثة بعمر 32 يوم .

Introduction

Gibberellic acid (a group of related called gibberellins) was discovered as a metabolic byproduct of the fungus *Gibberella fujikuroi*, (1). Gibberellins are one of the six major classes of plant growth regulators according to the American Society of Agricultural Science, (2). Gibberellic acid-3 (GA3) accelerates and improves yield of wide varieties of plants by increasing cell divisions, (3). Plant growth regulators enter animals body cells with their nutrition, and the most widely used in agriculture is GA3, (4). Several studies have suggested that GA3 has similar effect of some Asteroidial hormones such as Estrogen and Androgen, (5,6). Some other studies have shown fluctuated effect of it on the qualities of productivity in poultry. For example, (7) reported that positive effect of GA3 on body weights of poultry. (8) concluded that injection of GA3 itself or with supplementation of vitamin D3 to diets improve some of productive parameters especially the relative weights of eggshell and shell thickness of aged laying hens. On the other hand, GA3 has a negative effect on body of animals. Some studies demonstrated that animals chronic GA3 consumption increased tumor formation (9). It increases in Lipid Peroxidation such as malondialdehyde (MDA), and decreases in the level of antioxidant defense systems such as glutathione (GSH) for a group of rats that received 75 ppm of GA3 daily in drinking water for six weeks have reported, (10). Vitamin E is one of the most powerful soluble fat antioxidants. It protects cells and tissues from oxidative damage induced by free radicals (11), therefore it is the first line of protection against Lipid Peroxidation, (12).

This study aims to highlight positive and negative effects of Gibberellic acid on some productivity, physiological, and immunological parameters in broiler chicks, and the role of low level of vitamin E in reducing its negative impact.

Materials and methods

A total of ninety one day old chicks (Hubbard Classic) were brought from Al Shammari hatchery. These chicks were raised in a poultry field which belong to the college of Agriculture / University of Al-Qadisiya. Chicks were randomly distributed into 3 equal treatments (30 chicks for each treatment). They were fed on starting, growing and finishing rations. These rations were composed according to NRC 1994 (Table 1), (13). They were vaccinated by Newcastle vaccine (La sota strain) 2 times at 21 and 31 days age by spraying method. Chicks were weighted weekly. At both times of 32 and 49 days old measurements of: Dressing percentage, liver weight, humoral immunity against Newcastle disease using ELISA test, aspartate aminotransferase, and alanine aminotransferase concentrations were done. Biotic 8800 XL was used to measure antibody titers , and Reflotron Plus was used to measure AST and ALT.

Treatments of the experiment were arranged as follow: control treatment was the first which was given just plain tap water; Starting of 7 days, the second treatment was given water contains concentration of 150 ppm Gibberellic acid, and The third one was given water contains concentration of 150 ppm Gibberellic acid with concentration of 30 mg per liter vitamin E.

Tablets concentration of 10% GA3 that used in the experiment, was produced by Xiamen Vastland co. Ltd (china). It was obtained from the local markets of the city of Al-Diwaniyah. Vitamin E produced by Vapco Jordan Company was also used. Blood samples were taken during the slaughtering process and placed in 5 ml glass tubes that were anticoagulant free. After that, 2 ml special sealed glass tubes were used to isolate serum. These serum samples were preserved under 20 Celsius till laboratory analysis.

Data analysis was conducted according to analysis of variance method, which is the randomized completely block design (RCBD) using least significant difference (LSD) to compare the averages at $p \leq 0.05$ by Genstat computing program.

Table (1) Gross composition of experimental diet.

Ingredients (kg)	starting Period from 1 to 21 days age	Growing Period from 22 to 42 days age	Finishing Period from 43 to 49 days age
Maize	38	54.5	59.6
Soya bean meal	39.5	36.5	31.5
Wheat	14	-	-
Premix*	2.5	2.5	2.5
Oil	4.4	5.7	5.7
Limestone	1.3	0.5	0.4
Antibiotics and Antifungals	0.3	0.3	0.3
Total	100	100	100
Calculated Nutrient			
Crude Protein (%)	24	22	20
Energy (kcal)	3025	3150	3200
Calcium	%1	0.8 %	0.87 %
Phosphorus	0.4 %	0.35 %	0.4 %

* Premix containing vitamins (A, D3, E, K, B1, B2, B6, B12, Pantothenic acid, Biotin, Choline, Folic acid); minerals (Iron, Zinc , Copper , Manganese Cobalt , Selenium , Iodine) and Amino acid (Methionine).

Result and Discussion

Table (2) shows no significant differences in weekly weight among the treatments. Statistical increases were noticed in T2 as compared with T1 for all weeks. This agrees with what (7), which feeding 2-weeks old broiler chicks on diets containing GA3 (0, 1, 5, 25 and 125 ppm) for 3-weeks led to non-significant increase of body weight. Moreover, (14) observed that injecting five weeks old rabbits by GA3 dosed of (0, 50, 100, and 200 micrograms per kg of body weight) for four weeks leads to significant increases in body weight. There were statistical increases in T3 for the fifth, sixth and seventh weeks because of the role of vitamin E in cells and tissue protection from oxidative damage induced by free radicals, (11). (15) mentioned that there is a significant increase with weekly weight of birds exposed to thermal stress and feeding on ration containing high level of 0.6 g vitamin E / kg comparing with control group.

Table (2) Effect of Gibberellic acid and Gibberellic acid + Vitamin E Added to the drinking Water on the Rate of Weekly Weight.

Treatments	The Average of chicks Body Weight / gm					
	Week 2	Week 3	Week 4	Week 5	Week 6	Week 7
T1	360.0	787	1361	1900	2364	3271
T2	386.7	821	1375	1900	2483	3368
T3	386.7	773	1350	1929	2579	3471
L.S.D	N.S*	N.S	N.S	N.S	N.S	N.S

* NS = Not Significant at α =0.05.

Table (3) presents an increasing in the average of liver weights significantly at the second test as compared with the first test in both T2 and T3. This is normal because of organs and body

developments. According to the first test, average liver weight of T2 and T3 were statistically lower than the control. This is consistent with what (7) found, which when broiler chicks treated for period of 3 weeks with different concentration of GA3, liver weights were statistically lower than the control. On the other hand, T2 and T3 had significant increases as compared with T1 at 49 days old. This agrees with the observation of (16), which was that significant increase in liver weight as compared with the control when oral doses of 25, 50, and100 mg of GA3 / kg applied to mice with for 11 weeks. That increase might be due to the increases in glycogen liver contents, or to the GA3 effect that is similar to estrogen's effect on the liver weight, (6). Vitamin E had no involvement in liver weight changing .

Table (3) Effect of Gibberellic acid and Gibberellic acid + Vitamin E Added to the Drinking Water on the Rate of Liver Weight.

Treatments	Liver Weight at the First Test (gm)	Liver Weight at the second Test (gm)	Mean
T1	49.5	58.7	54.1
T2	44.3	75.1	59.7
T3	42.3	74.0	58.1
Mean	45.4	69.3	Among groups = N.S*
	Between Periods = 7.95		
L.S.D	Overlap = 13.78		

* NS = Not Significant at α =0.05.

According to Table (4), there are no significant differences among treated groups and control group at both 32 and 49 days old for the dressing percentage trait. It is clear that result indicates that there are statistical decline in the dressing percentage in T2 and T3 as compared with T1 at the 32 days old check. This agrees with what (7) found, which refers to decreasing in percentage of slaughter chicks weight when broiler chick fed on ration containing different ratios of GA3. Dressing percentage of T3 was statistically higher than other sets at the age of 49 days old. This is partly in line with what (15) agree on, which were no significant differences in dressing percentage between group that added 0.6 g vitamin E / kg to its diets and the control group (Both groups exposed to thermal stress).

Table (4) Effect of Gibberellic acid and Gibberellic acid + Vitamin E Added to the drinking Water on the Dressing Percentage.

Treatments	Dressing Percentage at the First Test %	Dressing Percentage at the Second Test %	Mean
T1	71.30	70.27	70.78
T2	69.32	70.68	70.00
T3	69.43	71.85	70.64
Mean	70.02	70.93	Among groups = N.S
	Between periods = N.S*		
L.S.D	Overlap = N.S		

* NS = Not Significant at α =0.05.

Table (5) shows immune status. Antibody titres of T2 was statistically higher than other treatments according to both checks (32 and 49 days old). This is consistent with the results of (17) who showed that GA3 is safe and efficacious for enhancing immune response against H5N1 antigen. Antibody titres against NDv of T3 statistically higher than the T1 at ages of 49 days old.

This is partly in line with what (18) found, vitamin E and selenium improves humoral immune system in broiler chickens against Newcastle disease virus. However, among ages, the antibody titers against Newcastle disease virus of the second and third sets have been raised statistically at 49 days old comparing with 32-days old. Overall, antibody titres mean of both tests of T2 was significantly higher than each of T1 and T3 means separately. This may be attributed to IL2, which: enhanced cytotoxicity of the macrophages and secretion of IL1, enhanced immunoglobulin synthesis and proliferation of B lymphocyte, enhancing proliferation of T lymphocyte and these two types play a major role in the humoral and cell mediated immunity of chicken (19). (17), reported that treated groups with low-dose (0.325 mg / L) and high-dose (0.650 mg / L) of GA3 showed an increasing in lymphocytes calculated cells and IL-2 production.

Table (5) Effects of adding Gibberellic acid and Gibberellic acid + vitamin E to the Broiler Chickens drinking water according to antibody titres of Newcastle disease using ELISA test.

Treatments	antibody titers at the First Test	antibody titres at the Second Test	Mean
T1	5740	5379	5560
T2	6926	10286	8606
T3	5178	6044	5611
Mean	5948	7236	Among groups = 1662.9
	Between periods = N.S*		
L.S.D	Overlap = N.S		

* NS = Not Significant at α =0.05.

Assessing the effectiveness of liver enzymes, aspartate aminotransferase and alanine aminotransferase, is an appropriate indication to assess the possible hepatic cells damage. These enzymes are two of some important indications in evaluation of liver functions. They indicate the abnormality of this organ, which could occur due to some differences in medical and physiological conditions, (20). Birds are considered abnormal when their AST enzyme activity in serum is higher than 320 IU / liter. Moderate damage had been noted in tissue in case of moderate increase of this enzyme up to 2 to 4 times that level. Moreover, tissue necrosis leads to more obvious increase in the enzyme activity, (21).

AST liver enzyme in table (6) shows that T1 was a significantly outperformed as compared with the second and the third sites at 32days old test. However, both of T2 and T3 significantly outperformed as compared with T1 at 49 days old test. In the same table, ALT check result showed that there is statistical increase in its level in T1 as compared with the second and third ones at the 32 days old test. ALT level was statistically higher in the T3 as compared with T1 and T2 at 49 days old test. This probably means that limited quantities of GA3 (150 ppm) have a positive effect on liver functions at age of 32 days. Unlike this, cumulative effect of it at 49 days old broiler chicken cause increasing in the concentration of these liver enzymes. This is consistent with the finding of (10), who pointed out that giving adult rats 75 ppm of GA3 (sub chronic toxicity) daily in drinking water for six weeks led to a significant increase in the level of ALT and AST. Results also shows that using both of vitamin E and GA3 in the third group led to ALT, AST concentration declined in at age of 32-days old. This might be attributed to the effectiveness of vitamin E in reducing the damage that occurs in the fatty acid by free radicals during the oxidative process, (22). Moreover, (23), reported that it helps to maintain the integrity of the membranes of liver cells. It plays role in preserving internal cellular components. While there was a significant increase of ALT for the group that fed with vitamin E compared with the control, there was non-significant differences of AST concentration for chicks fed on diets containing different levels of vitamin E, (24).

High ALT and AST concentration at the age of 49 days of the third group may be due to vitamin E amount limitation that used (30 mg per liter). Thus, it became enable to overcome the cumulative effect of GA3 at age of 49 Day. Comparing the two time test, AST level at 32 days old was statistically higher than 49 days old test of T1. On the other hand, AST concentration at 49 days old test was significantly higher than at 32 days in the second and third groups. In all groups, ALT at the age of 49 days was higher than its level at the age of 32. Generally, there were significant increases of the average levels of AST and ALT separately for the three groups at the first check comparing with the average of their levels of the second check. This is because that these enzymes get increased in case of liver tissue damage. So that avoiding this damage itself, contributes reducing the amount of these serum enzymes, (21).

Table (6) Effect of Gibberellic acid and Gibberellic acid + vitamin E to the drinking water on enzymes, AST and ALT.

Treatments	AST (U.I/l)			ALT (U.I/l)		
	32 Days old	49 Days old	Mean	32 days old	49 Days old	Mean
T1	597	517	557	10.10	11.82	10.96
T2	509	616	563	8.38	12.18	10.28
T3	453	619	536	7.86	13.66	10.76
Mean	520	584	Among T = N.S*	8.78	12.55	Among T = N.S
	Between periods = 50.4			Between periods = 2.724		
L.S.D	Overlap = 87.3			Overlap = N.S		

* NS = Not Significant at α =0.05.

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