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المجلة العراقية لبحوث السوق وحماية المستهلك

THE INFLUENCE OF SUPPLEMENTING ELLAGIC ACID TO BROILER DIETS ON PRODUCTIVITY AND WHITE BLOOD CELL DIFFERENTIAL COUNTS

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ABSTRACT

This study has been carried out in the animal field of the college of agricultural engineering sciences, university of Baghdad, for the period from 15/12/2021 to 26/1/2022 for 42 d, to investigate the effect of adding different levels of ellagic acid to the diet of broilers, on some physiological characteristics & oxidation indicators in meat compared to vitamin C in meat, 225 Ross 308 chicks were used, divided randomly to five treatments such us: T1: control group without additives to diet, & the other T2, T3, T4 was added ellagic acid (0.25, 0.5 and 0.75 mg/ kg diet) respectively and T5: was added vitamin C 0.5 mg/ kg, the birds were given ellagic acid & vitamin C from day one to the end of the experiment, the study results were showed a significant differences increase (P<0.05) improved at T4 treatment in the first & second week & in T2 and T4. in the 4th and 5th weeks treatment T2 was superior, to show a significant advantage of the control treatment & T1,T2 in the 2nd week for the trait of overweight, in the 4th and 5th weeks the treatment T2 was superior for the total weight gain, there was an improvement in the feed conversion in 4th week in T2, in the 5th week the treatment T2 was superior, there was a significantly superior in T2 for total weight gain rate, there was a significant increase in the feed conversion in T1 and T4 in the 2nd week.

Keywords: Broilers, ellagic acid, physiological characteristics, oxidative indicators.

دور اضافة حامض الايلاجك Ellagic acid وفيتامين C لعلائق فروج اللحم في الاداء الانتاجي وبعض الصفات الفسلجية والميكروبية ومؤشرا الاكسدة

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الخلاصة

أجريت هذه التجربة في حقل الطيور الداجنة التابع إلى قسم الإنتاج الحيواني/ كلية علوم الهندسة الزراعية/ جامعة بغداد للمدة من 2021/12/15 الى 2022/1/26 ولمدة 42 يوم وذلك لمعرفة تأثير إضافة مستويات مختلفة من حامض الايلاجك Ellagic acid الى عليقة فروج اللحم على بعض الخصائص الفسيولوجية ومؤشرات الأكسدة في اللحم، وتم استخدام 225 فرخ فروج لحم ROSS-308 بعمر يوم واحد غير مجنسة وزعت بشكل عشوائي إلى خمس معاملات وبواقع ثلاث مكررات لكل معاملة، اذ كانت المعاملة الأولى T1 عليقة سيطرة بدون اي إضافة، بينما كانت المعاملة الثانية T2 عليقة أساسية مضاف اليها 0.25 ملغم/ كغم حامض الايلاجك، والمعاملة الثالثة T3 عليقة أساسية مضاف اليها 0.5 ملغم/ كغم حامض الايلاجك، والمعاملة الرابعة T4 عليقة أساسية مضاف اليها 0.75 ملغم/ كغم حامض الايلاجك، في حين كانت المعاملة الخامسة T5 عليقة اساسية مضاف اليها 0.5 ملغم/ كغم فيتامين C، وأظهرت نتائج الدراسة حصول تفوق معنوي (P<0.05) للمعاملة T4 في الاسبوعين الأول والثاني، وتحسن في المعاملة T2 وT4 في الاسبوعين الرابع والخامس، كما تفوقت المعاملة T1 وT2 على باقي المعاملات بالنسبة لصفة الزيادة الوزنية في الأسبوع الثاني، كما وحصل تحسن معنوي لمعاملتي T1 وT4 بالنسبة لمعامل التحويل الغذائي في الأسبوع الثاني وحصل ارتفاع معنوي في المعاملة T2 في صفة الزيادة الوزنية كما وحصل تحسن معنوي في معامل التحويل الغذائي بالنسبة للمعاملة T1 وT4.

الكلمات المفتاحية: دجاج اللحم ، حامض الايلاجك، الخصائص الفسيولوجية، مؤشرات الأكسدة.

INTRODUCTION

Ellagic acid is a biologically active food polyphenol compound. It is one of the main antioxidants found in abundance in fruits and vegetables, it has many beneficial properties for health because it contains antioxidants **(Sun *et al.*, 2017),** such as anticancer **(Rahal *et al.*, 2014),** antibacterial **(Shahidi *et al.*, 2018)**, antimicrobial & anti-inflammatory **(Li *et al.*, 2015)** properties, the antioxidant effect is shown by deleting reactive oxygen and reactive nitrogen such as hydroxyl radicals, peroxyl, nitrite and peroxynitrite **(Seeram *et al.*, 2005),** and previous researches indicated that ellagic acid can remove free radicals **(Zheng *et al.*, 2020)**, preventing lipid peroxidation **(Kilic *et al.*, 2014)**. Therefore, there has been interested to understanding the role of polyphenols and their potential mechanisms of action in maintaining a healthy gut. and with the global ban on the use of antibiotics as growth promoters, there has been an impetus for efforts to use natural materials as feed additives tom proved the productive performance of broilers **(Duskaev *et al.*, 2018),** Therefore, the trend has been recently towards the use of materials extracted from plants as a medicinal alternative to improve the health of animals, which is called natural treatment. There is also clear evidence that adequate nutrition can help maintain a healthy gut **(He *et al.*, 2017; Wan *et al.*, 2018)**. However, gut health in young animals can easily deteriorate due to many factors, including disease-causing infection, inflammation and oxidation **(Liu, 2015; Wan *et al.*, 2021).** These factors lead to intestinal mucosal damage and dysfunction and thus adversely affect the growth performance and health of animals **(Liu *et al.*, 2008),** so the aim of this study was to show the effect of adding ellagic acid (as an antioxidant) to meatloaf ration on Its effect on the physiological performance and oxidation indicators of meat .

MATERIALS AND METHODS

This study was carried out in the animal field of the college of agricultural engineering sciences, university of Baghdad, for the period from 15/12/2021 to 26/1/2022 for 42 d, to find out the effect of adding different levels of ellagic acid to the diet of broilers, on some physiological characteristics & oxidation indicators in meat compared to vitamin C, 225 Ross 308 chicks were used, divided only to five treatments which were:

T1 without additives to diet (control),
T2: was added Ellagic acid at 0.25 mg/ kg diet
T3: was added Ellagic acid at 0.5 mg/ kg diet
T4: was added Ellagic acid at 0.75 mg/ kg diet
T5: was added vitamin C 0.5 mg/ kg.

Al the treatments gave ad libitum diet & water in the experiment period, the diet contents chosen as **National Research Council (1994)** which contain protein 23.14% in initiator diet & 21.6% in growth diet & 20.17% in final diet, while metabolism energy was 3001.00, 3103.7 and 3099.18 and 3204.89 Kcal/kg diet for each diet.

Soybean meal cake used an argentine source of crude protein content by 48% & 2440 Kcal/kg metabolism energy, protein meal user product from nether origin contain 40% crude protein 0.2117 kcal/kg protein metabolism energy 5% crude fat 5.00% crude fiber 2.81%, calcium 3.14%, phosphorus 2.65% lysine 3.85%, methionine 3.70%, methionine plus cysteine 4.12%, tryptophan 0.4%, threonine 1.80%. It contains a mixture of vitamins and minerals needed believes rare birds of these elements.

Blood samples and analysis

At the termination of the study, at 21 and 42 d of age, 9 birds per group totaling 3 birds per treatment replication, was removed randomly for blood collection. Blood samples (1 ML/bird) were collected from the ulnar is wing vein into EDTA tubes. Within two hours after

blood samples were collected, they were centrifuged (3000×g for 10 min at room temperature) to separate plasma from blood cells, and plasma was then decanted and stored in Eppendorf safe lock microcentrifuge tubes at -20°C until assayed.

Productive traits

Productive traits were measured according to the method of **Al-Faith & Najy (1989).**

Statistical analysis

Completely randomized design (CRD) was used to study the effect of different treatment in al traits, **(Duncan, 1955)** and multiple range tests was used to compare the significant differences between means. Data were analyzed by using statistical analysis system **(SAS, 2012).**

RESULTS AND DISCUSSION

We showed from (Table 1) the effect of adding ellagic acid and vitamin C to the broiler diet at different levels on body weight, as T4 outperformed T2 in the first week significantly ($P<0.05$), in the 2nd week a significant superiority ($P <0.05$) in T4 over T3 and T5, while in the 4th week T2 and T4 were significantly superior to T5 ($P<0.05$), and in the 5th week, T2 was superior to control T1 and T5, and T4 outperformed T5 significantly ($P<0.05$), and no significant differences were found in the mean weight of the beginning and the third week.

Table (1): Effect of adding ellagic acid on body weight of broilers compare with vitamin C (mean ± standard error).

Treatment	live body weight (g/bird)					
	Initial weight	Week 1	Week 2	Week 3	Week 4	Week 5
T1	43.60 ± 1.47	156.71 ± 2.34 ab	467.08 ± 13.09 ab	974.44 ± 24.52	1556.07 ± 7.39 ab	1998.56 ± 31.41 bc
T2	43.88 ± 0.76	147.06 ± 6.08 b	457.64 ± 14.83 abc	930.40 ± 30.79	1612.58 ± 9.75 a	2204.69 ± 22.82 a
T3	44.11 ± 1.19	149.46 ± 2.66 ab	427.51 ± 8.65 c	907.06 ± 31.77	1531.33 ± 52.69 ab	2074.49 ± 74.37 abc
T4	51.00 ± 6.66	160.00 ± 0.07 a	883.88 ± 9.62 a	969.68 ± 11.02	1588 ± 17.79 a	2144.78 ± 21.53 ab
T5	44.13 ± 0.86	154.71 ± 2.38 ab	443.08 ± 2.02 bc	938.66 ± 18.70	1469.40 ± 12.66 b	1948.13 ± 95.79 c
Significant	N.S	*	*	N.S	*	*

*: mean with different letters within the same column are significantly different from each other at significance ($P<0.05$).
N.S: no significant between treatments.

Noticed from (table, 2) showed the effect of adding Ellagic acid and vitamin C on the rate of weight gain of broilers, as there was a significant agreement ($P<0.05$) for the control T1, T2 and T4 over T3 and T5 in the second week, and in the fourth week, T2 outperformed T5. Significantly ($P<0.05$), but in the fifth week, T2 was significantly ($P<0.05$) superior to control T1, and for the overall weight gain rate, T2 was significantly superior to T1 and T5, while in the first week The third did not show significant differences.

Table (2): Effect of adding ellagic acid on the rate of weight gain of broilers compare with vitamin C (mean ± standard error).

Treatment	weight gain (g/bird)					Total weight gain
	Week 1	Week 2	Week 3	Week 4	Week 5	
T1	113.11 ± 0.87	310.37 ± 11.32 a	507.35 ± 15.35	581.62 ± 28.33 ab	442.48 ± 34.34 b	1954.96 ± 32.74 b
T2	103.17 ± 6.07	310.57 ± 8.75 ab	472.75 ± 27.26	682.17 ± 24.94 a	592.11 ± 14.62 a	2160.80 ± 23.30 a
T3	105.35 ± 1.47	278.04 ± 6.70 c	479.55 ± 31.49	624.26 ± 47.95 ab	543.15 ± 22.58 ab	2030.38 ± 73.81 ab
T4	109.00 ± 6.66	323.88 ± 9.66 a	485.80 ± 5.83	618.31 ± 17.04 ab	556.77 ± 14.63 ab	2093.78 ± 15.63 ab
T5	110.57 ± 3.71	288.37 ± 4.40 c	495.57 ± 20.69	557.73 ± 26.11 b	451.73 ± 84.10 ab	1904.00 ± 95.44 b
Significant	N.S	*	N.S	*	*	*

*: mean with different letters within the same column are significantly different from each other at significance ($P<0.05$).
N.S: no significant between treatments.

It was clear from (Table 3) that there are no significant differences between the control and the experimental treatments when adding different levels of Ellagic acid and vitamin C in the broiler's diet in the rate of feed consumption.

Table (3): Effect of adding ellagic acid on feed consumption of broilers compare with vitamin C (mean ± standard error).

Treatment	feed intake (g/bird/week)					Total feed consumption
	Week 1	Week 2	Week 3	Week 4	Week 5	
T1	119.10 ± 3.72	285.64 ± 1.19	504.02 ± 17.35	745.10 ± 19.37	1137.19 ± 8.54	2791.07 ± 35.98
T2	110.13 ± 12.13	308.57 ± 10.17	515.73 ± 1.13	765.24 ± 5.56	1172.35 ± 8.82	2872.04 ± 23.47
T3	110.21 ± 9.55	305.95 ± 13.53	502.30 ± 10.40	715.11 ± 3056	1163.77 ± 68.30	2797.77 ± 52.74
T4	105.46 ± 13.00	295.64 ± 14.60	503.04 ± 12.57	729.81 ± 8.36	1147.33 ± 25.67	2871.29 ± 24.31
T5	107.77 ± 0.64	297.46 ± 2.94	497.10 ± 11.52	733.28 ± 15.82	1127.55 ± 32.38	2763.18 ± 37.52
Significant	N.S	N.S	N.S	N.S	N.S	N.S

N.S: no significant between treatments.

In (Table, 4) there were a significant improvement ($P<0.05$) in the food conversion factor of T1 and T4 compared to T3 in the second week, while there were no significant differences between the control T1 and the rest of the experimental treatments.

Table (4): The effect of adding ellagic acid on the feed conversion of broilers compare with vitamin C (mean ± standard error)

Treatment	feed conversion (g feed / g weight gain)					Total feed conversion
	Week 1	Week 2	Week 3	Week 4	Week 5	
T1	1.05 ± 0.02	0.92 ± 0.03 b	0.99 ± 0.008	1.28 ± 0.08	2.60 ± 0.20	1.42 ± 0.02
T2	1.08 ± 0.17	0.99 ± 0.05 ab	1.09 ± 0.06	1.12 ± 0.04	1.98 ± 0.06	1.32 ± 0.02
T3	1.04 ± 0.08	1.10 ± 0.04 a	1.05 ± 0.08	1.16 ± 0.14	2.14 ± 0.05	1.38 ± 0.04
T4	0.96 ± 0.09	0.91 ± 0.06 b	1.03 ± 0.02	1.18 ± 0.02	2.06 ± 0.01	1.32 ± 0.01
T5	0.97 ± 0.03	1.03 ± 0.01 ab	1.00 ± 0.04	1.32 ± 0.06	2.69 ± 0.53	1.45 ± 0.06
Significant	N.S	*	N.S	N.S	N.S	N.S

*: mean with different letters within the same column are significantly different from each other at significance ($P<0.05$).
N.S: no significant between treatments.

Significant improvements in productive and physiological performance, such as: live weight was outperformed by T4 treatment in the first, second and fourth weeks, and T2 treatment in the fourth and fifth weeks, and the weight gain in T4 treatment in the second week & T2 treatment in the fourth and fifth weeks, & the rate of weight gain, may be due to that anti-oxidants Oxidation is critical to animal growth **(Lobo *et al.*, 2010),** The effect is shown Antioxidant by deleting reactive oxygen and reactive nitrogen species such as rhizomes hydroxyl, peroxyl, nitrite and peroxynitrite **(Seeram *et al.*, 2005),** Previous studies showed that ellagic acid can scavenge free radicals **(Zheng *et al.*, 2020),** and inhibits lipid peroxidation **(Kilic *et al.*, 2014).** or it may be because ellagic acid supplementation causes increased activity of digestive enzymes in the intestine and thus improves nutrient digestion, and intestinal absorption levels **(Amad *et al.*, 2011).** Ellagic acid can improve immune properties, activate enzymatic antioxidants, and control free radicals during stress conditions due to its antioxidant, antimicrobial, anti-inflammatory and anticancer properties **(Prakash & Prakash, 2011)**. This helps reduce pathogens in the intestines. Thus, nutrients can reach the intestinal lumen to be absorbed and converted to body mass **(Opara *et al.*, 2009).** These results are in agreement with the results obtained in this study, as the concentrations of AST and ALT were increased and the concentration of ALP decreased in the second treatment and the decrease of AST enzyme and ALT enzyme and the rise of ALP enzyme in the treatments T3 and T5, and

this may be due to the fact that ellagic acid supplementation increases the ability to digest nutrients on the dose basis, and that ellagic acid has a strong oxidative role, and is effective in protecting intestinal mucosal morphology and inhibiting the expression of intestinal inflammatory factors **(Sun *et al.*, 2017).** Since ellagic acid can prevent oxidation by acting as a scavenger of free radicals or delay the oxidation process indirectly, it may be due to the high accumulation of eellagic acid in the intestine and its ability to heal damage to the intestine and this may be related to the level of ellagic acid that accumulates in the intestinal epithelium and forms an antioxidant barrier against harmful oxidation products and thus protects other tissues **(Sun *et al.*, 2017)**, many indicated Studies have shown that antioxidants positively influence the digestion of nutrients **(Sahin *et al.*, 2003).** In many studies conducted, it was reported that active herbal compounds stimulate digestive enzymes secreted by the mucous membrane of the pancreas and intestines **(Platel & Srinivasan, 2000),** as it increases digestive enzymes in the pancreas such as amylase, lipase, trypsin and chymotrypsin **(Lee *et al.*, 2003)**, and increase digestion of the ether extract by increasing bile secretion, this leads to better digestibility of nutrients due to ellagic acid **(Harada & Yano, 1975).** We note from the (Table 5 and 6) that there was no significant difference in the number of white blood cells at the age of 21 and 42 d of the experiment.

Table (5): Effect of Ellagic acid supplementation compare with vitamin C on white blood cell count at 21 d of age (mean ± standared error).

Treatment	Traits					H/L ratio
	Lymphocyte	Monocyte	Basophile	Eosinophil	Heterophil	
T1	54.00 ± 6.51	8.00 ± 3.58	7.67 ± 4.76	9.33 ± 3.86	21.00 ± 4.51	0.417 ± 0.240
T2	26.00 ± 4.08	13.33 ± 6.60	22.00 ± 5.15	27.67 ± 5.73	11.00 ± 1.53	0.437 ± 0.189
T3	30.67 ± 5.91	15.00 ± 3.09	21.67 ± 3.03	18.33 ± 3.67	14.33 ± 1.76	0.472 ± 0.161
T4	44.33 ± 7.31	11.00 ± 5.52	14.00 ± 4.00	14.00 ± 3.00	16.67 ± 1.20	0.400 ± 0.174
T5	38.33 ± 6.26	16.00 ± 4.31	17.00 ± 2.31	15.67 ± 3.33	13.00 ± 2.08	0.335 ± 0.190
Significant	N.S	N.S	N.S	N.S	N.S	N.S

N.S: no significant between treatments.

Table (6): Effect of Ellagic acid supplementation compare with vitamin C on white blood cell count at 42 d of age (mean ± standard error).

Treatment	Traits					H/L ratio
	Lymphocyte	Monocyte	Basophile	Eosinophil	Heterophil	
T1	38.00 ± 3.00	11.67 ± 0.88	9.67 ± 2.03	16.33 ± 3.53	24.33 ± 3.84	0.639 ± 0.178
T2	41.00 ± 4.58	14.33 ± 2.19	15.67 ± 3.67	13.00 ± 3.61	16.00 ± 2.58	0.398 ± 0.135
T3	39.00 ± 1.15	14.67 ± 0.67	18.00 ± 2.58	16.67 ± 1.20	11.67 ± 2.88	0.300 ± 0.128
T4	44.00 ± 2.08	15.67 ± 1.20	12.67 ± 1.76	12.33 ± 2.03	15.33 ± 1.20	0.349 ± 0.126
T5	45.33 ± 2.40	13.00 ± 1.73	12.00 ± 2.58	15.00 ± 1.15	14.67 ± 1.45	0.329 ± 0.148
Significant	N.S	N.S	N.S	N.S	N.S	N.S

N.S: no significant between treatments.

CONCLUSIONS

Improvement in some productive traits in some weeks for live body weight and weight gain for T4 and T2 treatment. Also, acid additions did not affect the differential count of white blood cells.

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