

Mesopotamia Journal of Agriculture



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IMPACT OF DIETARY ADDITION OF NETTLE PLANT AS A NATURAL ANTIOXIDANT ON THE PHYSIOLOGICAL PERFORMANCE OF BROILER

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ABSTRACT

Article information Article history: Received: 22 /1 /2025 Accepted: 23 /6 /2025 Available: 30 /6/2025

Keywords:

broilers, corticosterone, glutathione, glutathione peroxidase, leptin.

DOI:<u>10.33899/mja.2025.156</u> 860.1529

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This study was conducted to investigate the nettle plant's physiological effects on the broilers' antioxidant status. At one day of age, 240 chicks (Ross 308) were randomly distributed in three groups (80 birds/group, weighing 40.6±0.3 g each), with four replications for each, and were reared until 42 days of age. The groups were as follows: 1st group (control): received a basal diet without addition; 2nd and 3rd groups: received a basal diet with 2.5 and 5 g nettle/kg diet, respectively. The addition of the nettle resulted in a significant (P≤0.05) increase in total antioxidants at the 5 g/kg diet dose. Similarly, levels of glutathione and glutathione peroxidase increased. On the contrary, the malondialdehyde decreased, and it was significant at the 5 g/kg diet dose, which led to a substantial decrease in corticosterone compared to the control group. Insulin-like growth factor-1 was not significantly affected by adding nettle, while leptin (P < 0.05) decreased significantly compared to the control group. On the other hand, nettle significantly improved the broiler's productivity, especially at the 5 g nettle/kg diet dose, as mean final body weight, total weight gain, and feed consumption significantly (P<0.05) increased. The feed conversion ratio significantly improved at 42 days of age compared to the control group. We included that the nettle plant can improve the physiological performance and the antioxidant status of broilers and may also reduce oxidative stress, thereby promote growth and improve feed conversion efficiency.

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INTRODUCTION

The intensive poultry production with fast-growing breeds and the rapid growth of global demand and consumption may lead to a decline in their health due to a decrease in their immune and physiological status and the emergence of problems in their productive performance, which may lead to major and numerous economic problems as a result of the stress and pathological factors that they were exposed to (Milosevic *et al.*, 2021 and Grzinic *et al.*, 2023).

Therefore, the trend was towards enhancing the bird's ability to withstand that intensive production, supporting its health, and maintaining a high level of productivity, taking into consideration the promotion of growth with natural enhancers that help the bird in challenging those factors that may decline its immunity and its productive performance, and staying away from using antibiotics (Korver, 2023 and Righi *et al.*, 2021). Today, there is a growing tendency to feed additives

that promote growth, such as prebiotics and probiotics (Kloor and Al-ubaidy, 2018), or some organic acids (Matty and Hassan, 2020) or vitamins (Rahawi *et al.*, 2022 a), or additives that may collect and contain all of that, which is meant by the natural medicinal plants and herbs and their extracts (Aminullah *et al.*, 2025 and Abd El-Hack *et al.*, 2022), as they are safe, relatively cheap, and available compared to others, which can be added to drinking water or mixed with ration (Sharma *et al.*, 2018 and Mustafa *et al.*, 2024).

Plant feed additives are a large group of plants with a rich content of various elements and compounds (Milosevic *et al.*, 2021 and Abdelli *et al.*, 2021) that have potential positive effects on animal health and productivity and thus on human health (Said *et al.*, 2015; Abdul-Majeed and Al-Krad, 2023 and Sadeeq *et al.*, 2024), and among these plants is the nettle plant.

Nettle, commonly known as stinging nettle (*Urtica dioica* L.), grows widely in our region (Mosul/ Iraq) (Abdul-Majeed *et al.*, 2021). It is a widespread wild plant that belongs to the Urticaceae family (Bhusal et al., 2022) and is used in folk medicine in many countries (Semalty *et al.*, 2017).

Nettle has immune and antioxidant properties because it contains polyphenolic compounds such as flavonoids and phenolic acids (gallic, ferulic, and caffeic acid) (Florez *et al.*, 2022), as well as tannins, terpenes, acetylcholine, histamine, serotonin, and formic acid (Abdelli *et al.*, 2021; Said *et al.*, 2015; and Devkota *et al.*, 2022). Also, it contains polyunsaturated fatty acids such as linolenic, linoleic, and palmitic acid (Bhusal *et al.*, 2022, and Taraseviciene *et al.*, 2023).

High in proteins, lipids, carbs, minerals, and vitamins are stinging nettle leaves (Said *et al.*, 2015 and Moula *et al.*, 2019), and they are a rich source of sodium, calcium, magnesium, manganese, and ferric (Abdelli *et al.*, 2021 and Florez *et al.*, 2022). They also contain vitamins A, C, K, B₁, B₂, pantothenic acid, and carotenoids (Said *et al.*, 2015 and Bhusal *et al.*, 2022).

Loetscher *et al.* (2013) used nettle leaves in layer diets as a feed additive due to their growth-promoting properties and antioxidant capacity. Nettle leaves contain antioxidant compounds, the most important of which are carvacrol and carvone (Farahani and Hosseinian, 2021). Sharma *et al.* (2018) mentioned that the addition of 2–4% nettles improved the growth performance and antioxidant status of broiler chickens, while Kregiel et al. (2018) showed that nettles have positive effects on improving the welfare and health status of broilers.

On the other hand, Farahani and Hosseinian (2021) mentioned that nettle can be used to treat heat stress; adding it at 4% reduced the levels of cortisol, AST, ALT, and creatine kinase and then improved the oxidative status of chickens exposed to chronic heat stress.

From those mentioned earlier, it turns out that the nettle plant has many uses because of the active substances it contains that affect the productivity of poultry. Therefore, our research aims to know the effect of nettle on the antioxidant status, lipid peroxidation, and level of stress hormone (corticosterone) in broiler chickens and their productive performance, with a call for additional research to investigate the effects of this plant due to the limited research work around it.

MATERIALS AND METHODS

Ethical approve

The study design and experimental chick treatments were approved by the Scientific Council in the Department of Animal Production and confirmed by the Animal Care and Ethics Board empowered by the Veterinary Medicine Faculty/University of Mosul with the number Um. Vet . 2024.059 dated 2/22/2024.

Experimental design

A total of 240-day-old Ross 308 broiler chicks were randomly distributed into three groups, each consisting of 80 birds, weighing 40.6±0.3 g each, and each group had four replicates (20 birds each). Chicks were weighed once a week until the trial concluded. At 42 days of age, the birds were slaughtered (8 birds/group), and blood samples were collected immediately during slaughter into plain tubes containing gel and a clot activator. The blood serum was then separated using a Kubota 5400 centrifuge at 3,000 rpm for 15 minutes and stored in Eppendorf tubes at -20°C until biochemical analysis was performed (Campbell, 1995). Broilers were raised in ground cages, and all the breeding requirements were provided for them according to the birds' age, such as temperature, humidity, and lighting. Water and feed were provided *ad libitum*, and the birds were fed according to the poultry nutrition requirements (NRC, 1994), as shown in Table 1.

Table (1): Broiler ration ingredients (starter and grower), crude protein, and metabolizable energy

Ingredients	Starter ration (From 1-21 days)	Grower ration (From 22-42 days)
	%	%
Yellow Corn	44	45
Wheat	11.45	14.25
Wheat Bran	4	4
Soybean Meal (44% protein)	35.5	30
Sunflower Oil	1.3	3
Protein Concentrate	2.5	2.5
Limestone (CaCO ₃)	0.5	0.5
Salt	0.25	0.25
Monocalcium Phosphate (MCP)	0.5	0.5
Total	100	100
Crude Protein (%)	22.0	20.0
Metabolizable Energy (kcal/kg)	2904	3060

Nettle leaves (*Urtica dioica* L.) were purchased and dried from local markets in Mosul city. They are well known in the city's folk medicine; then, they were crushed and mixed with the diet. The study groups are as follows: The first group (control group) was given a basal ration without additives. The second group was given a basal diet supplemented with 2.5 g of nettle/kg diet. The third group was given a basal ration supplemented with 5.0 g of nettle/kg diet.

The productive performance criteria were taken, which are: the chicks' initial and final body weights (IBW and FBW), total weight gain (TWG), feed consumption (FC), and feed conversion ratio (FCR).

The Antioxidant status parameters, including total antioxidant capacity (TAC), glutathione (GSH), glutathione peroxidase activity (GSH-Px), and malondialdehyde (MDA), were measured in serum using kits from Elabscience (USA), based on the principle of competitive ELISA. In addition, several hormones were measured, including leptin, insulin-like growth factor-1 (IGF-1), and corticosterone (CS). All serological analyses were performed using Elabscience kits (USA), based on the sandwich ELISA principle (Rahawi *et al.*, 2022 b and Abdul-Majeed *et al.*, 2022).

Statistical analysis

A one-way analysis of variance was used to perform statistical analysis on the collected data according to the completely randomized design (CRD) as described by Steel and Torrie (Steel and Torrie, 1960). Duncan's multiple range test was employed to assess the differences between group means at the probability level $P \le 0.05$ (Duncan, 1955) using the SAS statistical program (SAS, 2016).

RESULTS AND DISCUSSION

Due to its many properties, Nettle was used as a fodder additive for poultry (Loetscher *et al.*, 2013). It is a natural antioxidant and growth promoter, as it increases weight and improves the feed conversion ratio of birds fed on it (Upton, 2013).

It appears in Table (2) that the dietary addition of the nettle plant significantly (P≤0.05) increased total antioxidant levels at a dose of 5 g/kg diet (16.03 U/ml) in compared to the control group (11.23 U/ml), and in the same direction was the effect of nettle on the level of glutathione and glutathione peroxidase, as both increased significantly when giving nettle plant 2.5 and 5 g/kg diet compared with the control

Table (2): Means (±SE) effect of dietary nettle plant on the serum antioxidant status of broilers at 42 days of age.

Groups	Control	2.5 g nettle/kg diet	5 g nettle/kg diet
TAC (U/ml)	11.23±0.37 b	13.53±0.98 ab	16.03±0.91 a
GSH (µmol/L)	0.351±0.01 c	0.437±0.02 b	0.561±0.02 a
GSH-Px (μmol/L)	66.20±0.77 b	69.64±0.90 a	69.37±0.77 a
MDA (ng/ml)	26.17±1.96 a	20.83±1.86 ab	17.83±1.64 b

A significant difference between various letters in the same row at $P \le 0.05$.

TAC= total antioxidant capacity; GSH= glutathione; GSH-Px= glutathione peroxidase activity; MDA= malondialdehyde.

group. On the contrary, the MDA level decreased when the nettle plant was given and was significant ($P \le 0.05$) at a dose of 5 g/kg diet (17.83 ng/ml) compared to the control group (26.17 ng/ml) for broilers at 42 days of age.

From Table 2, we see an improvement in the antioxidant status of the broiler's serum at 42 days of age. The total antioxidants increased significantly when adding 5 g nettle/kg feed (16.03 U/ml) compared to the control group (11.23 U/ml).

The data presented in Table 2 confirm and reinforce our previous statement, as both glutathione and glutathione peroxidase (GSH-Px) levels increased

significantly in the blood of broilers supplemented with 2.5 and 5 g/kg of nettle compared to the control group. Conversely, malondialdehyde (MDA) levels were significantly reduced, particularly at the 5 g/kg dose, where the concentration decreased to 17.83 nmol/ml compared to 26.17 nmol/ml in the control group.

The researchers Devkota *et al.* (2022) and Upton (2013) stated that the phenolic compounds of nettle, such as carvacrol and carvone, may protect the body's cells from free radicals. This is in addition to its vitamins (A, C, E, B₁, B₂), which play an additional role in protecting the body from lipid oxidation, which leads to an elevated level of the TAC values (Devkota *et al.*, 2022).

From this, we conclude that nettles can reduce the level of oxidative stress and enhance the antioxidant status of broiler chickens at the age of 42 days. Farahani and Hosseinian (2021 and 2022) mentioned this when they gave nettles to broilers. The nettle plant is a natural antioxidant and growth promoter, increases weight, and improves the feed conversion ratio of birds fed on it (Upton, 2013).

From Table 3, it is clear that the IGF-1 level was not affected by adding the nettle to the diet, while the leptin level significantly declined in both doses of nettle at $P \le 0.05$. For the corticosterone level, it decreased significantly ($P \le 0.05$) (120.04 ng/ml) when 5 g/kg of diet nettles were administered, compared with control group (163.73 ng/ml).

Table (3): Means (\pm SE) effect of dietary nettle plant on broilers' serum corticosterone, IGF-1, and Leptin at 42 days of age.

Groups	Control	2.5 g nettle/kg diet	5 g nettle/kg diet
CS (ng/ml)	163.73±7.57 a	141.86±5.03 ab	120.04±6.20 b
IGF-1 (pg/ml)	39.79±1.04 a	38.64±1.56 a	39.12±1.79 a
Leptin (pg/ml)	726.98±31.60 a	523.17±24.85 b	435.58±16.66 c

A significant difference between various letters in the same row at $P \le 0.05$. CS= corticosterone; IGF-1= insulin-like growth factor-1.

From Table (3), we see that the level of the stress hormone (corticosterone) decreased significantly by increasing the nettle dose to 5 g/kg of feed compared with the control group, and this indicates that the nettle has antioxidant activity and works to reduce the level of oxidative stress in the body because it contains phenolic substances such as carvacrol and carvone (Farahani and Hosseinian, 2021and 2022), which improved the antioxidant status, as can be seen in Table (2), as it significantly increased the level of TAC, GSH, and GSH-Px while significantly reduced the level of lipid peroxidation index (MDA), as also reported by Jaiswal and Lee (Jaiswal and Lee, 2022).

Also, from Table 3, we note that the level of IGF was not affected by adding nettles to the feed, and this indicates that it maintained its level and the level of tissue growth and development. This is because IGF-1 has insulin-like effects due to the similarity of its protein molecular structure to that of insulin (Laron, 2001), as it is mainly produced in the liver, skeletal muscles, and many other tissues in response to growth hormone stimulation, which works with it side by side in promoting the normal growth and development of body tissues (Lodjak *et al.*, 2014 and Sinpru *et al.*, 2021).

It is clear from Table (3) that nettle at a dose of 2.5 and 5 g/kg of diet decreased significantly the level of leptin hormone, which led to (especially at a dose of 5 g of nettle/kg of diet) an increase in feed consumption and final weight and as well as an enhancement in the FCR as compared to the control group, as shown in Table (4). Leptin, a polypeptide hormone secreted from adipose tissues, regulates appetite and energy balance in the body (Taouis and Dridi, 2001). When its level rises, it reduces food intake in broilers and vice versa (Denbow *et al.*, 2000).

On the other hand, we note from Table 4 that the addition of nettles at a dose of 5 g/kg diet led to a significant increase in the FBW (2719 g) and TWG (2679 g) compared with the control group (2539 and 2499 g), respectively, at 42 days of age.

Table (4): Means (\pm SE) effect of dietary nettle plant on broiler growth traits at 42 days of age.

Groups	Control	2.5 g nettle/kg diet	5 g nettle/kg diet
IBW (g)	40.25±0.29 a	40.92±0.46 a	40.70±0.40 a
FBW (g)	2539±41.37 b	2639±45.38 ab	2719±36.02 a
TWG (g)	2499±41.12 b	2598±45.62 ab	2679±36.34 a
FC (g)	3812±47.27 b	3894±69.49 ab	4072±74.70 a
FCR*	1.63±0.04 a	1.50±0.03 b	1.43±0.02 b

A significant difference between various letters in the same row at $P \le 0.05$.

IBW= initial body weights; FBW= final body weights; TWG= total weight gain; FC= feed consumption; *FCR= feed conversion ratio (g diet/g weight gain).

In the same direction, we notice from Table 4 that the addition of nettle increased feed consumption (FC) significantly ($P \le 0.05$) (4072 g) compared with the control group (3812 g). Also, we note that the FCR has improved dramatically in both added doses of nettle (1.50 and 1.43 g diet/g weight gain) compared with the control group (1.63 g diet/g weight gain) for broilers at 42 days of age.

Table 4 explained that the addition of nettle, especially the dose of 5 g/kg of feed, led to a significant increase in the FBW, TW, and FC, which led to an enhancement in the FCR compared to the control group. Perhaps the increase in feed consumption was associated with a decrease in the level of leptin Table 3), which decreased and led to a feeling of hunger and an increase in appetite (Taouis and Dridi, 2001), and thus the feed consumption increased. This led to an increase in FBW and an improvement in FCR at 42 days compared to the control group Table 4.

On the other hand, it can be stated that the antioxidants, vitamins, and other elements present in the components of the nettle plant improved the body's physiological status and stimulate the beneficial bacteria growth in the digestive system of poultry (Abdul-Majeed *et al.*, 2021 and Babinszky *et al.*, 2021), resulting an increase in the digestion of nutrients such as proteins, carbohydrates, and lipids (Behboodi *et al.*, 2021), and that the components of nettle, especially the antioxidants, led to the protection the epithelial cells of the intestine from oxidative stress (Miller, 2001), which led to the growth of the epithelial cells, which then benefited from food very well and promoted growing the birds (Jaiswal and Lee, 2022).

CONCLUSIONS

This study concludes that the inclusion of nettles, especially 5 g/kg feed, can lead to an improvement in the antioxidant status and a decrease in the level of oxidative stress in broilers, which then enhances the body's growth and feed conversion ratio. Nevertheless, we call for more research on this plant when it is given to broilers due to its many benefits and the lack of research conducted on it.

ACKNOWLEDGMENT

The authors acknowledge the University of Mosul's College of Agriculture and Forestry for its help in carrying out this investigation. We also thank Dr. Saeb Younis Abdul-Rahman for his accurate and helpful advice on the study.

CONFLICT OF INTEREST

This is a declaration that the authors have no conflicts of interest.

أثر الإضافة الغذائية لنبات القريص كمضاد أكسدة طبيعي في الأداء الفسلجي لفروج اللحم

غدير عبدالمنعم محمد رحاوي 1 ، وائل ذنون يونس 2 ، عبدالله فتحي عبدالمجيد 1 قسم الإنتاج الحيواني / كلية الزراعة والغابات / جامعة الموصل / الموصل / العراق 2 فرع الطب الباطني / كلية الطب / جامعة الموصل / الموصل / العراق 2

الخلاصة

أجريت هذه الدراسة لمعرفة التأثيرات الفسلجية لنبات القريص في حالة مضادات الأكسدة لفروج اللحم، إذ وزع عشوائيا 240 فرخا (روز 308) بعمر يوم واحد في ثلاث مجموعات (80 طائرا/مجموعة، وزن كل منها $40.6 \pm 0.34 \pm 0.0$ غرام) بواقع أربع مكررات لكل منها، وربيت لغاية عمر 42 يوما. كانت المجموعات وفق الآتي: المجموعة الأولى (مجموعة سيطرة): أعطيت عليقة أساسية بدون إضافات، المجموعتان الثانية والثالثة: أعطيتا عليقة أساسية مضافا إليها 2.5 و 5 غم نبات القريص/كغم علف على التوالي. أدت إضافة نبات القريص إلى عليقة أساسية معنوية (أ ≤ 0.05) في مستوى مضادات الأكسدة الكلية عند جرعة 5 غم/كغم علف، وبالمثل، ارتفعت مستويات الكلوتاثيون والكلوتاثيون بيروكسيديز، بينما على العكس من ذلك، انخفض مستوى المالوندايالديهايد وكان معنويا عند جرعة 5 غم قريص/كغم علف، مما أدى إلى انخفاض معنوي في هرمون الكورتيكوستيرون مقارنة مع مجموعة السيطرة. لم يتأثر معنويا مستوى عامل النمو الشبيه بالإنسولين – 1 بإضافة نبات القريص، بينما انخفض معنوياً (أ ≤ 0.00) مستوى في الأداء الإنتاجي لفروج اللحم ولاسيما جرعة ال 5 غم/كغم علف، إذ ارتفع معنوياً (أ ≤ 0.00) معدل وزن الجسم النهائي والزيادة الوزنية الكلية وكمية العلف المستهلكة، وتحسن معنوياً معامل التحويل الغذائي عند عمر 42 يوما مقارنة مع مجموعة السيطرة. نستنتج من ذلك أن نبات القريص يمكن أن يحسن الأداء الفسلجي وحالة مضادات الأكسدة لفروج اللحم، وقد يقلل أيضاً من الإجهاد التأكسدي، ومكان أن يحسن الأداء الفسلجي وحالة مضادات الأكسدة لفروج اللحم، وقد يقلل أيضاً من الإجهاد التأكسدي، وبالتالي يعزز النمو وبحسن كفاءة التحويل الغذائي.

الكلمات المفتاحية: اللبتين، فروج، كلوتاثيون، كلوتاثيون بيروكسيديز، كورتيكوستيرون.

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