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Impact of Aqueous Extract of Borage Powder on Biochemical, Immunological, and Microbial Characteristics of Poultry Birds

Mohammed Hussein Abadi[1]*, Haider Qasim Baqer[1], Haifa Ali Awahd[2]

[1]Department of Animal Production, College of Agriculture, University of Karbala, Karbala, Iraq; [2]Department of Food Sciences, College of Agriculture, University of Karbala, Karbala, Iraq.

Abstract | This experiment aimed at establishing the impact of adding aqueous extract of borage powder in the drinking water on biochemical, immunological, and microbial properties among poultry farms. A total of 120 one-day old, unsexed broilers (Ross308) with an initial weight of 40 grams were used in the study. The chicks were randomly subjected to four experimental treatments and each treatment contained 30 chicks and three replicas (10 chicks/replica). Different treatments were applied where the control group (no additives) was left untreated and in the group T2, T4 and T3 were supplemented with an aqueous extract of borage flower powder at concentration of 10, 20 and 30 ml/liter of water, respectively. The experimental findings revealed a high level of significant improvement ($p<=0.05$) in blood images of treatment T3 than the other treatments in respect to the HDL trait. The value of high-density lipoproteins (HDL) was lowest in control treatment, whereas the value of cholesterol, low density lipoproteins (LDL), very-low-density lipoproteins (VLDL), and triglycerides in the blood serum was lowest in treatment T3 and highest in the control treatment. The T3 treatment and the other supplementation treatments significantly improved ($p\leq0.05$) the blood protein concentrations of albumin, globulin, and total protein as compared to the control treatment. The supplementation treatment however registered an increment in AST and ALT in the supplementation treatments as opposed to the control treatment. The logarithmic counts of E. coli bacteria increased significantly, and the logarithmic counts of the lactobacillus bacteria increased significantly ($p\leq0.05$) in both the jejunum and ileum contents at the T3 treatment and other supplementation treatments than the control treatment. The relative weight of Fabricius gland and spleen improved significantly ($p\leq0.05$) when the T3 treatment was administered and when the other treatments of supplementation were applied compared to the control treatment. Taken together, these finding highlight a positive impact of aqueous extract of borage powder on the performance and productivity of poultry.

Keywords | Borage plant, Productive traits of broiler chickens, Immune traits, Microbial traits, Blood count

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***Correspondence** | Mohammed Hussein Abadi, Department of Animal Production, College of Agriculture, University of Karbala, Karbala, Iraq; **Email:** mohammed.abadi@uokerbala.edu.iq
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INTRODUCTION

The population growth in most countries during the last half of the 20th century has been significant, leading to an increase in demand for poultry products, including eggs and meat (Al-Jebory *et al.*, 2024; Ajafar *et al.*, 2024a, b). This is due to their high nutritional value, low fat content, and ease of digestion and palatability compared to red meat. The poultry sector is considered one of the fastest growing agricultural sectors due to the rapid turnover of capital

and significant industrial progress in both developed and developing countries (Food and Agriculture Organization of the United Nations, FAO, 2021). Poultry projects are of great importance to achieving economic growth and sustainable development in developing countries. Therefore, broiler farming has become known as the broiler industry (Ahizo *et al*., 2024). A top priority for the poultry industry has been identifying nutritional supplements that can enhance the health of the organism in terms of quantitative and qualitative output (Gerzilov *et al*., 2015).

Medicinal plants have been used for centuries to treat many ailments naturally, and now phytotherapy is emerging as a complementary method to traditional drug treatment for many ailments (Pieczak *et al*., 2012). One such plant is borage (*Borago officinalis* L.), an annual plant with blue flowers native to the Mediterranean Basin. It was introduced to Europe, Asia, Africa, and South America. In Greek medicine, *Borago officinalis* L. is employed to activate the brain, external and internal senses, activate the nerves, activate the heart, and even the respiratory system. It is also used to treat liver and biliary related issues, skin diseases and others (Gani, 2011). Sharma (2003) it is a common herb highly grown in the form of leaves, flowers and seeds, which are commercially used in their nutritional and medicinal properties. The flowering portion is regarded to be edible, and it is rich in bioactive compounds, which are valuable in advancing the health of humans. The chemical studies conducted on the plant show that it has a therapeutic use in traditional medicine and is typical in botany and traditional remedy all over the world (Ramezani *et al*., 2019).

It has been observed in the previous research that crude extracts of borage include various significant bioactive compounds, including, but not limited to, quercetin, rutin, and spatulinol among others (Ibrahim and Duha, 2023). Flavonoids, terpenes, alkaloids, saponins and coumarins are the biological active compounds and included in the list are alkaloids, phenols, steroids and terpenoids among other compounds as byproducts of toxicology, environmental and therapeutic significance. Greek medicine has been applied to the *Borago officinalis* L. to stimulate the brain, internal and external senses, nerves, heart, respiratory systems, liver and biliary problems, skin diseases, and other diseases (Gani, 2011). It has been shown to possess pharmacological effects and biological actions such as antioxidant, antimicrobial, anticancer, anti-inflammatory, insecticidal and genotoxic actions. Its properties are associated with the high bioactivity of bioactive compounds that are extracted in different parts, such as leaves, flowers, seeds, and roots (Nakaa *et al*., 2024). The borage plant aqueous extract is an antioxidant (Karimi *et al*., 2018). It is important because it is able to eliminate free radicals in living cells. Active compounds have the ability to shield cells against damage that is caused by metabolic activities that cause excessive oxidation (Karimi *et al*., 2013). According to Sobhanirad (2022), borage powder is a better choice compared to a traditional diet devoid of additives regarding its impact on the immune system and the number of blood cells in obese chicks. The evidence proved that the BP2 and BP1 systems promote the increase of the number of white blood cells. These findings show that the supplementation of borage powder enhances immunity in broilers chicken.

Medicinal herbs and plants that contain flavonoid, vitamin C, and carotenoid are useful to the immune system of both animals and humans. These substances are immune-boosting agents, just as is the case with garlic. According to (Abedin *et al*., 2014), borage powder supplemented into the diet of male broilers chicken at a dosage of 5 g per kg and 7.5 g per kg enhanced the immune system of chicken, leading to an increase in the number of white blood cells. They also included borage extract in three groups of broilers in another study by (Sebhani *et al*., 2022) at the dose of 150 ml per liter, 300 ml per liter, and 600 ml per liter. The findings indicated that BE in the concentration of 600mg per liter was useful in countering the effects of high-density stress. They further proposed BE as a nutrition supplement to improve the performance of the birds during stressful conditions. Kast (2004) established that medicinal herbs used as poultry feed aid in enhancing immunity by increasing the population of white blood cells. Moreover, borage flower extract has been demonstrated possessing a antibacterial effect, when the agar disc method is used against *Bacillus subtilis* and *Staphylococcus monocytogenes*. Singh *et al*. (2017) added that borage aqueous extract can fight against *Pseudomonas* and *Escherichia coli*. This implies that borage flower extract has the ability of fighting with a number of viruses, bacteria, fungi and toxins because of its active ingredients which include saponins, titanins and alkaloids.

This study was conducted to identify how the aqueous borage extract can affect the drinking water of broilers on certain hematological and microbial parameters of broilers.

MATERIALS AND METHODS

Preparation of the aqueous extract of borage flower powder

The dried borage flowers were purchased at one of the Karbala Governorate markets and crushed in a small electric grinder into a fine powder. The extract was subsequently prepared as suggested by Hermandez *et al*. (1994). Briefly, 1.0 g of dry powder was mixed with 2 ml/liter of distilled water using an electric mixer. The solution was left to stand after 24 hours at room temperature. This solution was then incubated in 60 C water bath over one hour and filtered

using a number of layers of sterile medical gauze to make it useable in the experiment.

Chick management and feeding

The chicks were kept in 2 three-story cages each occupancy of the floor being 1 x 2 m. The floor was divided into two sections that had area of 1 x 1 m. There were 10 broiler chicks in each cage. Gas incubators and electric heaters were used to improve the incubation of the hall to keep the temperatures and was done with an electronic thermometer within the days of raising to the age of marketing (37) days. The broiler chicks were given all the conditions to develop and grow, and the growth of food and water was facilitated. It was made up of a lighting system 24 hours a day in the first three days of life of the chicks, one hour of darkness being allowed at the days of rearing, in order to accustom the chicks and to prevent their disturbance and congestion in the current sector of electricity.

The birds were fed at will and a plastic feed dish of diameter 40 cm was used in the first week, with each chick fed in one dish and then that was slowly substituted with the circular plastic feeders in the rearing system. Water was made ready and offered in inverted plastic troughs of a capacity of 5 liters over the rearing period. The chicks were given ready-made rations (Pellet feed pellets) that had been prepared by the Al-Waha Factory in the Governorate of Karbala and contained three rations, namely the first ration was the starter ration that was used at the age of 1-14 days, second ration at the age of 15-28 days, and the third ration at the age of 29 days to the end of the experiment, which lasted 37 days in total.

Physiological characteristics

The 37-day chickens were sampled in the jugular vein and placed in tubes that lacked anticoagulant. The blood was centrifuged at 3,000 rpm/15min. The serum was stored in various sterile tubes at -18 °C, and tested in laboratory. Measures were then made on total protein, total cholesterol, and triglycerides (HDL, LDL, and LDL). Riyadh Hino Specialized Laboratory of analysis of pathology in the Holy city of Karbala did the analyses.

The level of total protein in chicken serum was also determined using the procedure adopted by Tietz (1999). The method of Tietz (1999) was used to follow the enzymatic hydrolysis of cholesterol in the serum of the birds. The enzymatic hydrolysis method was used to identify the concentration of triglycerides in the serum of birds as detailed in the method of Tietz (1999). The method of measuring HDL concentration in bird serum utilizing the enzymatic hydrolysis approach was based on the method proposed by Burstein *et al.* (1980) VLDL was also determined based on the technique used by Tietz (1999). Spleen and bursa of Fabricius were weighed on a sensitive balance prepared in China after being detached. Their weights were then compared and computed.

Serum Antibodies Volumetric Standard Calculation of liver enzyme levels (Alanine, Aspartate, Aminotransferase, Glutathione, and Malonodialdehyde) (MDA, GSH, AST, ALT) was done. Six random samples of each treatment were taken, and two samples of each replicate, one of them was a jugular vein sample at the age of 35 days. The blood was put in 10 ml glass tubes without the use of anticoagulant and tilted to eliminate the clot. A centrifuge of blood samples with a speed of 4000 rpm was used to take 10 minutes. The laboratory tests on the serum were prepared as per instructions on the kit by storing the serum in sterile tubes at -18 °C. The samples were put in a refrigerated box and dispatched to determine the concentration of liver enzymes, antioxidants and the antioxidants (MDA, GST, ALT, AST) in the serum by using a kit supplied by the French company (Biolabo). This test was carried out as per the company instructions in the Lutein Laboratory (a commercial lab). To analyse veterinary labs in the Har Saghir area (Salman *et al.*, 2025; Arkan *et al.*, 2025). Microbial tests were done in accordance with (APHA, 1978).

Statistical analysis

The effect of various treatments on the studied traits was studied using a completely randomized design (CRD). Duncan (1955) multinomial test was used to compare significant differences between means at the 0.05 and 0.01 levels of significance. The SAS (2001) program was used for statistical analysis according to the following mathematical model:

$$Yij = \mu + Ti + eij$$

RESULTS AND DISCUSSION

Effect of using aqueous extract of borage plant on biochemical blood characteristics of lipid profile

Table 1 indicate the impact of borage flower powder aqueous extract on the hematological and biochemical parameters of the blood of broiler chicken. Treatment T3 was significantly better in the HDL trait than the other treatments. The lowest high-density lipoprotein (HDL) was registered in the control treatment. The T3 treatment had the lowest levels of cholesterol, low-density lipoprotein (LDL), very-low-density lipoprotein (VLDL), and triglyceride concentration in the blood serum whereas the control treatment had the largest levels of these substances in the blood profile (Duha and Ruaa, 2023).

Table 1: The effect of using the aqueous extract of borage plant on the lipid profile (cholesterol concentration, high-density lipoproteins (HDL), low-density lipoproteins (LDL), very-low-density lipoproteins (VLDL) and triglyceride concentration) in the blood serum of broiler ± standard error.

Treatments	Cholesterol	HDL	LDL	VLDL	Triglyceride
T1	89.92±0.10A	57.21±0.15d	22.78±0.24a	9.941±0.03a	1.606±0.013a
T2	86.31±0.19C	63.93±0.05b	15.51±0.26c	6.87± 0.06c	1.732±0.010c
T3	84.59±0.31D	65.36±0.22a	13.14±0.52d	6.07±0.14d	1.881±0.008d
T4	87.03±0.05B	59.59±0.21c	18.77±0.13b	8.66±0.03b	1.691±0.008b
Significant	*	*	*	*	*

Table 2: Effect of using aqueous extract of borage plant on the concentrations of albumin, globulin, total protein, AST, ALT, and pH in the blood serum of broiler (mean ± standard error).

Treatments	Albumen	Globulin	Total protein	AST	ALT	pH
T1	2.197±0.016B	1.728±0.016d	3.925±0.030d	27.90±0.100d	16.55±0.041d	7.156±0.017b
T2	2.266±0.009B	2.198±0.015b	4.464±0.024b	28.96± 0.024b	17.41±0.008b	7.216±0.012a
T3	2.358±0.023A	2.369±0.021a	4.727±0.035a	29.51±0.008a	18.16±0.031a	7.226±0.012a
T4	2.219±0.046B	1.901±0.014c	4.120±0.041c	28.68±0.031c	17.20±0.093c	7.193±0.008ab
Significant	*	*	*	*	*	*

Effect of using aqueous borage extract on the concentrations of albumin, globulin, total protein, AST, ALT, and pH in the blood serum of broiler chickens.

As shown in Table 2, the addition of aqueous extract of borage flower powder to drinking water changed blood characteristics. The level of albumin demonstrates that there is a very great variation between the treatment T3 and the remaining treatments, and that the treatment T3 has the highest level of albumin. The two addition treatments, T2 and T4 and the control treatment T1 have no significant difference. The concentration of globulin reflects a large variation among all the treatments with all addition treatments showing better results compared to the control treatment. Treatment T3 was more effective as compared to treatment T2 and T4 and the control treatment T1. T1 had the lowest concentration recorded in control treatment. In addition, the total protein concentration shows that all the treatments have significant differences where addition treatments are better as compared to the control treatment, treatment T3 is better as compared to the addition treatments T2 and T4, and the control T1. The (AST) concentration exhibits a high variation among all treatments, all the addition treatment has performed better than the control treatment and T3 better than T2 and T4, and the control treatment T1, respectively. Regarding (ALT), it shows that there also exist significant differences among all treatments, where all addition treatments are better than control treatment, with treatment T3 being better than the addition treatments T2, T4, and control treatment T1. The (AST) concentration exhibits a high variation among all treatments, all the addition treatment has performed better than the control treatment and T3 better than T2 and T4, and the control treatment T1, respectively. Lastly, the hydrogen provides a notable difference involving the addition treatment T4, T2 and T4, on the other hand, the addition treatment T3 and T2 were significantly higher than the control treatment T1, and the addition treatment T4 did not achieve a significant difference on the control treatment T1 (Haider, 2024).

Tables 1 and 2 indicate a significant superiority in most concentrations in favor of the T3 treatment of beneficial compounds and substances in the blood serum over all treatments and the treatment record without adding the lowest level. The superiority is due to the addition treatments, especially the T3 treatment and the last treatment, an aqueous extract of borage flowers, 20 ml/liter of drinking water, which decreased the concentration of cholesterol. These results were consistent with (Cheraghi *et al*., 2023), which was conducted on carp fish when adding borage powder to their feed, which led to a significant increase in the protein content in the blood serum of fish that ate a diet with added borage powder compared to the control group. Significant differences were also found in the levels of albumin and globulin in fish that fed feed with added borage powder. Malekzadeh *et al*. (2018) have demonstrated that the use of medicinal herbs, such as sage powder, added to broiler feeds contributed to a decrease in cholesterol, triglycerides, and low-density lipoprotein (LDL) levels in broiler meat, while the addition of sage powder significantly increased high-density lipoprotein (HDL) levels. The study (Taleghani *et al*., 2015) also confirmed that the effect of adding thyme extract to broiler chickens led to a significant decrease in total blood cholesterol and triglyceride levels. The response of total blood protein, albumin, and globulin to different levels of

DTE was significantly higher than in the control group. There were no changes in blood calcium, phosphorus, and uric acid levels, as well as in the activity of liver enzymes such as ALT, AST, and ALP. These results are consistent with Haider (2024) in his study on feeding birds an aqueous extract of cloves, which improved the birds' hematological characteristics.

Effect of using a borage aqueous extract on some immune traits

The effect of using a borage aqueous extract on the relative weight of the fabricia gland and spleen in broiler.The effect of adding a borage flower powder aqueous extract to drinking water is outlined in Table 3. The relative weight of the Fabricia Gland was significantly higher for treatment T3 than for both the addition treatments and the control treatment T1. The relative weight of treatment T2 was significantly higher than for treatment T4 and the control treatment T1. No significant difference was recorded between treatment T4 and the control treatment T1. The relative weight of the spleen was significantly higher between treatment T1, the control treatment having the lowest relative weight, and between the addition treatments. The T4 treatment outperformed the control treatment, and T3 outperformed T4. No significant difference was recorded between treatment T3 and T2, and no significant difference was recorded between treatment T2 and T4. These results are consistent with what was found Ehsan and Saeid (2022) in his study of the effect of borage on bird nutrition, where the addition of an aqueous extract of borage improved productive traits and enhanced the immune response of broiler chickens. According to the results, the use of BE at 600 mg/l is useful in reducing the effects of density stress. Therefore, BE can be used as a nutritional strategy to improve the bird performance in stressful conditions. These results are consistent with those of Ehsan and Saeed (2024) in their study on the effects of borage on bird nutrition. According to their findings, using 600 mg/L of borage extract (BE) is beneficial in reducing the effects of intense stress, improving productive performance, and enhancing the birds' immune response.

Table 3: Effect of using a borage aqueous extract on the relative weight of the fabricia gland and spleen in broiler (mean± standard error).

Treatments	Gland of fabricius relative weight	Spleen relative weight of
T1	0.0687±0.0017c	0.1037±0.0023c
T2	0.0907±0.0031b	0.1130±0.0017ab
T3	0.1023±0.0017a	0.1157±0.0025a
T4	0.0767±0.0039c	0.1093±0.0025b
Significant	*	*

Effect of using aqueous borage extract on microbial characteristics, including logarithmic numbers of *Lactobacilli* and *E. coli* (cfu/gr), in the intestinal contents of broiler chickens

Analysis of finding shown in Table 4 indicate the effect of adding aqueous extract of dried borage flower powder to the drinking water of broiler chickens on logarithmic numbers of *Lactobacilli*. There were significant differences between treatments, with all addition treatments outperforming the control treatment, and treatment T3 outperforming all treatments. Treatments T4 and T2 did not record significant differences, with the control treatment recording the lowest numbers. As for E. Coli, there were significant differences between all treatments, with treatment T1 outperforming the control treatment over all treatments, treatment T4 outperforming T2, and treatment T3 outperforming T3, with T3 achieving the lowest numbers.

Table 4: Effect of using aqueous extract of borage plant on the logarithmic numbers of *Lactobacilli* and *E. coli* (cfu/gr) of the intestinal contents of broiler (mean ± standard error).

Treatments	*Lactobacilli*	*E. coli*
T1	3.142± 0.038 c	9.869 ± 0.078 a
T2	3.923 ± 0.012 B	8.994 ±0.074 c
T3	4.187 ± 0.067 a	8.386 ± 0.024 d
T4	3.815 ±0.073 b	8.494 ± 0.014 b
Significant	*	*

Table 4 indicated the effect of using aqueous extract of borage plant on the logarithmic numbers of *Lactobacilli* and *E. coli* (cfu/gr) in the intestinal contents of broiler chickens. *Lactobacilli bacteria* in the intestinal contents of broiler chickens outperformed the control treatment in all addition treatments, and treatment T3 outperformed treatments T2 and T4, respectively. The logarithmic numbers of *E. coli* bacteria in the intestinal contents of broiler chickens were outperformed in the control treatment in all addition treatments. Singh *et al.* (2017) have also indicated that borage flower extract acts as an antibacterial against bacteria such as *Pseudomonas* and *E. coli*. This extract also has an effective effect against some viruses, bacteria, fungi, and toxins because it contains saponins and other active ingredients (Al-Saeedi *et al.*, 2021; Alsudani *et al.*, 2025).

CONCLUSIONS

In conclusion, adding the aqueous extract of borage flower powder to drinking water had a significant superiority on the improvement in the relative weight gain of the

Fabricius gland and spleen. Additionally, anincrease in the logarithmic count of beneficial Lactobacilli and a decrease in the count of harmful pathogenic *E. coli* bacteria in the jejunum and ileum was noticed.

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NOVELTY STATEMENT

The novelty of this research lies in understanding the effect of the aqueous extract of borage on the hematological, mycobacterial, and histological characteristics of birds, using it at new concentrations not previously used in research, administered via the birds' drinking water.

AUTHOR'S CONTRIBUTION

MH conducted the practical experiment of raising chicks, fulfilling all their requirements. HQ collected, edited, and organized the data, including tables and Immunological and biological tests a analyzed the results statistically. HAA discussed the results and wrote the research paper.

Generative AI and AI-assisted technology statement

The authors declare that no Genrative AI was used in the creation of this manuscript.

Conflict of interest

The authors have declared no conflict of interest.

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