

Effect of adding different levels of Shilajit compared to Vitamin E on some blood parameters in Broiler

Shadan Mohammed Hadi¹

Nihad Abdul-Lateef Ali²

College of Agriculture/AL-Qasim Green University-Animal Production Department-Iraq.

¹ Email: shadan.mohammed@agre.uoqasim.edu.iq

²Email: dr.nihad@agre.uoqasim.edu.iq

Abstract

This experiment was conducted in the poultry farm of the Department of Animal Production, College of Agriculture, Al-Qasim Green University for the period 3/11/ 2025 to 17/12/ 2025. The study aimed to effect of Shilajit supplementation in drinking water and diets of broiler on productive performance, physiological and immune responses compared with vitamin E . 360 unsexed (Ross 308) broiler chicks from Al-Anwar hatchery were used, Divided randomly to with 8 experimental treatments for each treatment 45 birds, and each treatment included three replicates for each replicate of 15 birds. The experimental treatments were as follows:

T1 was Control group which was free from any addition, T2 based on adding vitamin E a concentration of 250 mg/ Kg feed. Treatments (T3, T4, T5) added the aqueous extract of Shilajit at a dose of (1, 2, 3) ml/liter of drinking water and at a concentration of 1%, Treatments (T6, T7,T8) added of Shilajit at levels (1,2,3) g/Kg feed . The results are as follows:

T3,T4 T5 T7, and T8 recorded a significant improvement ($P\leq 0.05$) in the number of white blood cells compared with the other treatments. Meanwhile, all Shilajit supplementation treatments, in addition to the Vitamin E treatment, showed a significant decrease ($P\leq 0.05$) in the percentage of heterophils (%) and the H/L ratio compared with the first treatment (control). Regarding the percentage of lymphocytes (%), the third, fourth, fifth, sixth, and eighth treatments showed a significant improvement ($P\leq 0.05$) compared with the first treatment (control).

The results of the experiment also showed a significant improvement ($P\leq 0.05$) in total protein concentration in the fourth, fifth, seventh, and eighth treatments. Moreover, all supplementation treatments recorded a significant increase ($P\leq 0.05$) in albumin concentration compared with the first treatment (control). It was also observed that all supplementation treatments showed a significant decrease ($P\leq 0.05$) in uric acid concentration compared with the first treatment (control).

All Shilajit supplementation treatments recorded a significant improvement ($P\leq 0.05$) in the concentration of the hormone Triiodothyronine (T3) compared with the first treatment (control). As for the concentration of the hormone Thyroxine (T4), all supplementation treatments showed a significant improvement ($P\leq 0.05$) compared with the first treatment (control).

Key words: shilajit, Physiological Parameters , vitamin E ,broiler.

Introduction

The poultry industry is considered one of the fastest-growing and most widespread sectors within agriculture globally, playing a pivotal role in developing countries as a primary source of animal protein. Poultry meat is characterized by its high nutritional value and is regarded as the best source of high-quality protein [1,2]. Consequently, several international companies specialized in poultry production have focused on conducting crossbreeding and genetic improvement programs to achieve rapid growth rates. This has resulted in commercial broiler strains with productive performance surpassing that of previous decades, albeit with a shorter lifespan. This situation necessitates a greater reliance on enhancing the immune aspect by increasing disease resistance and reducing the stress induced by rapid growth [3,4]. [5] observed that natural antioxidants inhibit free radical activity and mitigate the effects of oxidative stress resulting from rapid growth.

Shilajit is considered a natural dietary supplement containing a complex mixture of organic acids, such as fulvic acid, minerals, and phenolic compounds possessing antioxidant and immune-enhancing properties [6]. Studies have indicated that

Shilajit holds significant importance in human nutrition, contributing to improved nutritional biochemistry, supporting the immune system, and reducing biological stress markers [7]. In the poultry field, an experiment conducted by [8] demonstrated that supplementing Shilajit in broiler diets led to a notable improvement in live body weight, feed conversion ratio, and feed intake compared to the control treatment. Regarding vitamins, certain ones like Vitamin E act as antioxidants and are effective in reducing stress induced by rapid growth [9]. Vitamin E functions under stressful conditions to protect the body from the harmful effects of free radicals [10]. Many workers in the agricultural product processing sector experience stress related to broiler production. This, in turn, leads to decreased immunity. Therefore, many specialists have worked to mitigate this effect by using natural antioxidants [11].

The study aimed to :To investigate its role in regulating physiological and immune functions, To examine its antioxidant activity, To determine the most effective method of administration, whether via drinking water or feed and To identify the optimal concentration.

Materials and Methods

The experiment was conducted at the Poultry Farm, College of Agriculture, Al-Qasim Green University, from 13/11/ 2025 to 17/12/ 2025. The experiment aimed to study the effect of Shilajit supplementation in drinking water and feed on the Physiological Parameters of broilers compared to Vitamin E 95%, using Ross 308 broiler chicks. The birds were reared on a bedding of white wood shavings at a thickness of 7 cm. Feed was provided ad libitum. Chicks were fed a starter diet (23% crude protein and 3000 kcal metabolizable

energy/kg feed) from one day of age until the end of the third week. Subsequently, it was replaced with a grower diet (21.5% crude protein and 3100 kcal metabolizable energy/kg feed) until the end of the fifth week. Table (1) shows the composition of the starter and grower diets used in feeding the chicks throughout the experiment period and their calculated chemical composition. A total of 360 unsexed one-day old Ross 308 broiler chicks with an average initial body weight of 39 g were used in the experiment. The chicks were randomly

distributed among the treatments, with 45 chicks per treatment divided into three replicates per treatment (15 chicks/replicate). There were 8 treatments as follows: T1: control group (no supplementation). T2: supplementation with Vitamin E at 250 mg/kg feed. T3: supplementation with 1 ml of aqueous Shilajit extract (1% concentration) / L of drinking water. T4: supplementation with 2 ml of aqueous Shilajit extract (1% concentration) / L of drinking water. T5: supplementation with 3 ml of aqueous Shilajit extract (1% concentration) / L of drinking water. T6: addition of 1 g Shilajit / kg feed. T7: addition of 2 g Shilajit / kg feed. T8: addition of 3 g Shilajit / kg feed.

The Shilajit was mixed manually with the feed gradually until the feed was homogeneously mixed.

Image (1) illustrates the Shilajit material used in the experiment. The experiment included the study of the following traits: hematological parameters, biochemical traits, and the measurement of leptin and thyroid hormones at 35 days of age. A completely randomized design (CRD) was used to study the effect of different treatments on the studied traits. Differences between means were compared using Duncan's multiple range test [12]. The ready-made statistical program SAS [13] was used.

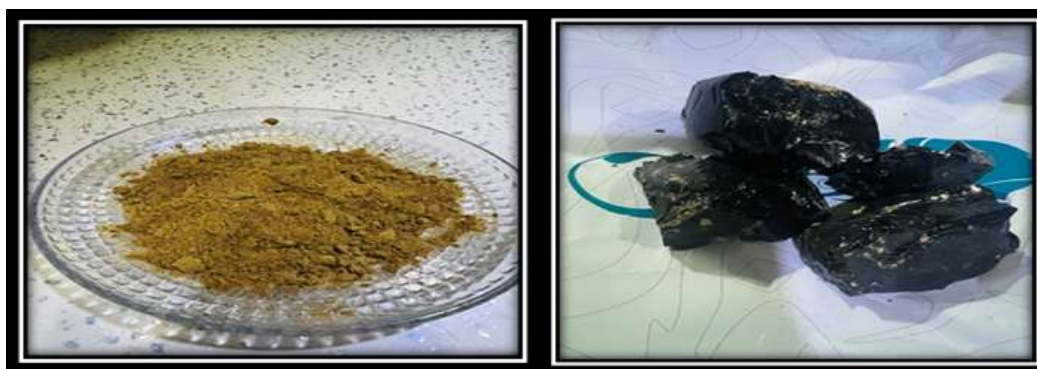


Image (1) the Shilajit material used in the experiment

Table 1: Percentages of feed ingredients used in the experiment and their chemical composition

Feed Ingredients	Starter Diet % (Days 1-21)	Grower Diet % (Days 22-35)
Yellow corn	38.50	42.29
Soybean meal (44%)	44.36	40.11
Wheat	10	10
Protein concentrate	2.5	2.5
Dicalcium phosphate	0.04	0.0
Calcium carbonate	1.26	1.07
Vegetable oil (sunflower)	2.29	3.15

Sodium chloride	0.17	0.17
Additives (vitamins and mineral salts)	0.5	0.5
DL-Methionine 99%	0.21	0.15
L-Lysine	0.09	0.01
Threonine	0.08	0.05
Total	100	100
**Calculated chemical analysis		
Metabolizable energy (Kcal/kg)	3000	3.100
Crude protein %	23	21.5
Crude fat	4.17	3.91
Calcium	0.96	0.78
Phosphorus	0.58	0.56
Sodium	0.16	0.16
Digestible lysine	1.44	1.29
Digestible methionine	0.77	0.70
Digestible threonine	0.97	0.88
Digestible methionine + cysteine	1.08	0.99
Dietary electrolyte balance	236.23	220.74
Dry matter %	89.51	89.84
Lipids %	3.10	3.08
Crude fiber %	1.02	1.09

(1)Broiler protein concentrate manufactured by Proyene / Dutch company. ** Chemical analysis was calculated based on [14], according to the ROSR (2022) company table, and according to the USDA (2018) program (American).

Results and Discussion

Table (2) shows the effect of adding Shilajit to drinking water and broiler diets and its comparison with Vitamin E on some cellular blood parameters of broiler chickens at 35 days of age (mean \pm standard error). Regarding the number of white blood cells ($\times 10^3/\text{mm}^3$ of blood), the results of the statistical analysis indicated that the third, fourth, fifth, seventh, and eighth treatments recorded a significant improvement ($P \leq 0.05$) in the number of white blood cells compared with the first (control), second, and sixth treatments. The second and sixth treatments also showed a significant increase ($P \leq 0.05$) in the number of white blood cells compared with the first treatment (control), which recorded the lowest number of white blood cells ($28.33 \times 10^3/\text{mm}^3$ of blood).

As for the percentage of heterophils, all supplementation treatments recorded a significant decrease ($P \leq 0.05$) in the heterophil percentage compared with the first

treatment (control), which recorded the highest heterophil percentage (29%).

Regarding the percentage of lymphocytes, the third, fourth, fifth, sixth, and eighth treatments showed a significant improvement ($P \leq 0.05$) compared with the first (control) and second treatments. However, the seventh treatment did not show any significant differences compared with the other experimental treatments.

Concerning the heterophil to lymphocyte ratio (H/L), all supplementation treatments recorded a significant decrease ($P \leq 0.05$) in the H/L ratio compared with the first treatment (control), which recorded the highest ratio (0.47). The third, fourth, and fifth treatments recorded the lowest H/L ratio with a significant difference ($P \leq 0.05$) compared with the other experimental treatments.

Table 2 Effect of Shilajit supplementation in drinking water and feed of broilers, compared with Vitamin E in some hematological traits of broiler chickens at 35 days of age (mean \pm standard error)

Treatment	Studied Traits			
	WBC $10^3/\text{mL}^3$ blood	Hetrophilis Percentage %	Lymphocytes Percentage %	Percentage H/L
T1	28.33 \pm 0.13 c	29.00 \pm 1.15 a	61.00 \pm 1.05 b	0.47 \pm 0.05 a
T2	30.50 \pm 0.28 b	26.00 \pm 0.37 b	62.00 \pm 0.52 b	0.41 \pm 0.01 b
T3	32.31 \pm 0.32 a	22.00 \pm 0.28 c	65.00 \pm 0.63 a	0.33 \pm 0.05 c

T4	32.83 ± 0.16 a	19.62 ± 0.33 d	64.36 ± 0.38 a	0.30 ± 0.04 c
T5	32.41 ± 0.30 a	20.00 ± 0.87 d	64.42 ± 1.16 a	0.31 ± 0.06 c
T6	30.52 ± 0.25 b	25.00 ± 0.55 b	64.09 ± 1.40 a	0.39 ± 0.07 b
T7	33.16 ± 0.74 a	24.17 ± 1.45 bc	63.10 ± 2.08 ab	0.38 ± 0.05 b
T8	32.16 ± 0.16 a	24.43 ± 0.66 bc	65.23 ± 1.19 a	0.37 ± 0.06 b
Level of significace	*	*	*	*

***Means within the same column bearing different superscripts differ significantly (P<0.05)**

T1: control group (no supplementation). T2: supplementation with Vitamin E at 250 mg/kg feed. T3: supplementation with 1 ml of aqueous Shilajit extract (1% concentration) / L of drinking water. T4: supplementation with 2 ml of aqueous Shilajit extract (1% concentration) / L of drinking water. T5: supplementation with 3 ml of aqueous Shilajit extract (1% concentration) / L of drinking water. T6: addition of 1 g Shilajit / kg feed. T7: addition of 2 g Shilajit / kg feed. T8: addition of 3 g Shilajit / kg feed.

The significant improvement in hematological parameters, represented by the increase in white blood cell count and lymphocyte percentage, as well as the significant decrease in heterophil percentage and the H/L ratio in all Shilajit supplementation treatments, whether in

drinking water or in the diet, compared with the first treatment (control), may be attributed to the fact that Shilajit is rich in minerals such as zinc and iron. These minerals contribute to the activation of the immune system and stimulate the formation of blood cells in tissues, which leads to an increase in the production of white blood cells and enhances the body's defense capability [15,16].

This improvement may also be attributed to the high content of antioxidants in Shilajit, such as phenolic compounds, flavonoids, and vitamins including vitamin C, which act to suppress free radicals and thereby maintain the normal physiological status of the body [17]. In contrast, Shilajit treatments recorded a significant improvement in lymphocyte percentage compared with the first treatment (control) and the Vitamin E treatment. This is considered a positive indicator of improved humoral and cellular immune responses in broiler

chickens, as lymphocytes play a key role in regulating immune responses. Their increase may be attributed to the immunostimulatory effect of Shilajit due to its content of bioactive compounds such as organic compounds and other constituents that enhance immune cell activity and improve immune system efficiency [18].

Regarding the significant improvement observed in the Vitamin E treatment in white blood cell count and the significant reduction in heterophil percentage and H/L ratio compared with the first treatment (control), this may be attributed to the vital role of Vitamin E as a fat-soluble antioxidant. It protects the cellular membranes of blood cells, enhances their stability, and prevents their destruction caused by free radicals, as well as preventing lipid peroxidation in the phospholipids composing the cell membrane. This helps maintain the integrity and function of blood cells and increases their biological efficiency [19]. Moreover, Vitamin E contributes to improving immune system efficiency by increasing immune cell activity, enhancing immune responses, and reducing stress effects in broiler chickens [20], which ultimately leads to an increase in white blood cell numbers and a decrease in heterophil percentage and H/L ratio, an important indicator of reduced stress levels in birds [21].

Table (3) shows the effect of adding Shilajit to drinking water and broiler diets and its comparison with Vitamin E on the biochemical parameters of blood serum in

broiler chickens at 35 days of age (mean \pm standard error). Regarding total protein concentration (g/100 ml), the fourth, fifth, seventh, and eighth treatments recorded a significant improvement ($P \leq 0.05$) compared with the first treatment (control), which recorded the lowest total protein concentration (4.31 g/100 ml) and did not differ significantly from the second, third, and sixth treatments.

As for albumin concentration (g/100 ml), all supplementation treatments, including the second treatment (Vitamin E supplementation), recorded a significant improvement ($P \leq 0.05$) compared with the first treatment (control), which recorded the lowest albumin concentration (2.16 g/100 ml). Regarding globulin concentration (g/100 ml), no significant differences were observed among all experimental treatments. Similarly, the results of the statistical analysis for uric acid concentration (mg/100 ml) showed that the first treatment (control) recorded the highest uric acid concentration with a significant difference ($P \leq 0.05$) compared with the other treatments. The fifth, seventh, and eighth treatments recorded the lowest uric acid concentrations without significant differences ($P \leq 0.05$) compared with the second, third, and sixth treatments.

With respect to glucose concentration (mg/100 ml), the fifth treatment recorded a significant decrease ($P \leq 0.05$) compared with the first treatment (control), which recorded the highest glucose concentration (384.52 mg/100 ml), and this treatment did not differ significantly from the other experimental treatments.

Table 3 Effect of Shilajit supplementation in drinking water and feed of broilers, compared with Vitamin E Biochemical parameters of broiler chicken blood serum at 35 days of age (mean \pm standard error)

Treatment	Studied Traits				
	Total protien g/100mL	Albumin g/100mL	Globulin g/100mL	Urice acid mg/100MI	Glucose mg/100mL
T1	4.31 ± 0.57 b	2.16 ± 0.07 b	2.14 ± 0.39	6.67 ± 0.23 a	384.52 ± 28.06 a
T2	4.81 ± 0.16 ab	2.64 ± 0.12 a	2.17 ± 0.19	5.11 ± 0.14 bc	356.79 ± 6.30 ab
T3	4.93 ± 0.12 ab	2.86 ± 0.03 a	2.07 ± 0.12	5.08 ± 0.18 bc	366.61 ± 10.59 ab
T4	5.48 ± 0.15 a	2.91 ± 0.04 a	2.57 ± 0.32	5.02 ± 0.11 b	361.64 ± 14.57 ab
T5	5.80 ± 0.11 a	2.88 ± 0.06 a	2.92 ± 0.27	4.63 ± 0.23 c	352.58 ± 3.30 b
T6	4.84 ± 0.21 ab	2.76 ± 0.09 a	2.08 ± 0.18	5.18 ± 0.33 bc	357.99 ± 12.72 ab
T7	5.43 ± 0.39 a	2.72 ± 0.05 a	2.71 ± 0.44	4.16 ± 0.13 c	362.32 ± 14.77 ab
T8	5.32 ± 0.35 a	2.74 ± 0.07 a	2.58 ± 0.24	4.25 ± 0.17 c	368.16 ± 11.47 ab
Level of significace	*	*	N.S	*	*

*Means within the same column bearing different superscripts differ significantly (P<0.05)

NS: not significant

T1: control group (no supplementation).
T2: supplementation with Vitamin E at 250 mg/kg feed. T3: supplementation

with 1 ml of aqueous Shilajit extract (1% concentration) / L of drinking water. T4: supplementation with 2 ml of aqueous Shilajit extract (1% concentration) / L of drinking water. T5: supplementation with 3 ml of aqueous Shilajit extract (1% concentration) / L of drinking water.

ISSN 2072-3857

T6: addition of 1 g Shilajit / kg feed.

T7: addition of 2 g Shilajit / kg feed.

T8: addition of 3 g Shilajit / kg feed.

Total protein is considered an essential indicator of poultry health, as it plays a major role in tissue formation, transport of vital substances, and support of enzyme and hormone functions. Total protein also plays a crucial role in enhancing body immunity through the production of antibodies and the repair of damaged tissues, thereby supporting rapid growth and cellular regeneration. Furthermore, measuring total protein helps in evaluating the protein status of the body and its capacity for production and tissue repair [22]. Based on the results of the present study, it can be stated that the Shilajit treatments (fourth, fifth, seventh, and eighth) led to an increase in total protein concentration compared with the first treatment (control). This may be attributed to the presence of humic acids in Shilajit, particularly fulvic acid, in addition to several mineral elements that improve digestive efficiency and nutrient absorption, thereby enhancing the utilization of amino acids. Consequently, this promotes protein synthesis in the liver and increases its level in blood serum. These compounds also have the ability to reduce inflammation in the intestinal lining and maintain the integrity of the intestinal mucosal membrane, thus creating a more favorable environment for nutrient absorption. In addition, they stimulate digestive enzymes such as pepsin and trypsin through the secretion of intestinal and pancreatic juices and reduce oxidative damage. Moreover, these compounds stimulate the synthesis of hepatic proteins such as albumin and globulin and improve protein metabolism and liver function [23]. This improvement is reflected in the overall health status of the flock and the

reduction of oxidative stress caused by free radicals, which is represented by the increased concentration of total protein.

Albumin is one of the major proteins in blood serum and plays an essential role as a source of amino acids required for the synthesis of tissue proteins, especially during the rapid growth period in birds. Albumin serves as a reserve of amino acids that support physical growth, particularly under dietary limitations ([24]. The significant improvement in albumin concentration observed in all Shilajit treatments as well as the Vitamin E treatment compared with the first treatment (control) may be attributed to the increase in total protein concentration. An increase in total protein concentration in blood serum indicates an increase in protein synthesis and a decrease in protein degradation [25]. Albumin represents the largest protein fraction in the blood and functions in the transport of carbohydrates, fatty acids, vitamins, and some mineral elements [26].

Regarding uric acid, which represents the final product of protein catabolism in birds [27], the decrease in uric acid concentration observed in all supplementation treatments compared with the first treatment (control) may be attributed to the presence of numerous biologically active compounds in Shilajit powder, such as flavonoids, phenolic acids, alkaloids, and vitamins including vitamin E [28,29]. The mechanism by which these bioactive compounds exert a uric acid-lowering effect can be summarized in their antioxidant activity, which plays an important role in reducing oxidative stress, eliminating free radicals, and protecting proteins from degradation, thereby preventing their conversion into glucose through the process of gluconeogenesis under the

influence of corticosterone hormone [30].

In addition, the presence of vitamin C in Shilajit may have contributed to lowering uric acid concentration in blood serum of the Shilajit treatments through increasing glomerular filtration and renal reabsorption. Both vitamin C and uric acid are reabsorbed through an anion exchange mechanism in the proximal convoluted tubule.

The potential reasons for increased glomerular filtration include the antioxidant effect of vitamin C in enhancing the supply of microvascular blood vessels in the glomeruli, which increases blood flow, dilates afferent arterioles, and enhances reabsorption along with ions such as sodium and potassium [7].

Regarding the decrease in serum glucose concentration in birds of the fifth treatment compared with the first treatment (control), this may be attributed to the presence of chlorogenic acid in Shilajit powder, which stimulates pancreatic beta cells and enhances the secretion of greater amounts of insulin in response to elevated blood glucose levels. It also increases glucose metabolism through the glycolysis pathway and promotes the entry of glucose into the plasma membrane [31].

Table (4) illustrates the effect of adding Shilajit to drinking water and broiler diets and its comparison with Vitamin E on the concentration of leptin hormone and thyroid hormones (T3 and T4) in blood serum of broiler chickens at 35 days of age (mean \pm standard error). The results of the statistical analysis indicated that there were no significant differences among all experimental treatments in leptin hormone concentration (ng/ml).

Regarding the concentration of the thyroid hormone Triiodothyronine (T3) (ng/ml), treatments three, four, five, six, seven, and eight recorded a significant improvement ($P \leq 0.05$) compared with the first treatment (control), which recorded the lowest concentration of T3 hormone (1.43 ng/ml) without a significant difference ($P \leq 0.05$) compared with the second treatment.

As for the concentration of the thyroid hormone Thyroxine (T4) (ng/ml), all supplementation treatments recorded a significant improvement ($P \leq 0.05$) compared with the first treatment (control), which recorded the lowest concentration of T4 (8.17 ng/ml), whereas the eighth treatment recorded the highest concentration of the hormone (13 ng/ml).

Table 4 Effect of Shilajit supplementation in drinking water and feed of broilers, compared with Vitamin E in the concentration of leptin and thyroid hormones in the serum of broiler chickens at 35 days of age. (mean \pm standard error)

Treatment	Studied Traits		
	Lipten Ng/mL	Throiiidothyronin e (T3) Ng/mL	Thyroxine (T4) Ng/mL
T1	2.28 ± 0.10	1.43 ± 0.12 B	8.17 ± 0.18 f
T2	2.39 ± 0.39	1.69 ± 0.24 Ab	9.33 ± 0.21 e
T3	2.21 ± 0.30	2.47 ± 0.21 A	11.00 ± 0.40 cd
T4	2.58 ± 0.35	2.52 ± 0.18 A	11.48 ± 0.42 bc
T5	2.74 ± 0.26	2.55 ± 0.23 A	12.24 ± 0.16 ab
T6	2.35 ± 0.33	2.12 ± 0.17 A	10.09 ± 0.33 de
T7	2.52 ± 0.27	2.32 ± 0.14 A	11.92 ± 0.24 bc
T8	2.43 ± 0.35	2.40 ± 0.25 A	13.00 ± 0.36 a
Level of significace	N.S	*	*

*Means within the same column bearing different superscripts differ significantly (P<0.05)

NS: not significant

T1: control group (no supplementation).
T2: supplementation with Vitamin E at

250 mg/kg feed. T3: supplementation with 1 ml of aqueous Shilajit extract (1% concentration) / L of drinking water. T4: supplementation with 2 ml of aqueous Shilajit extract (1% concentration) / L of drinking water. T5: supplementation with 3 ml of

ISSN 2072-3857

aqueous Shilajit extract (1% concentration) / L of drinking water.
T6: addition of 1 g Shilajit / kg feed.

The hormones Triiodothyronine (T3) and Thyroxine (T4) are among the main hormones secreted by the thyroid gland. Both play vital roles in regulating metabolism and energy balance in the body, as well as controlling cellular metabolic rates, which influence growth processes, muscle development, and energy utilization. These hormones are considered metabolically active and help cells regulate the utilization of lipids, carbohydrates, and proteins in poultry. In addition, T3 and T4 play an important role in rapid growth and the ability to adapt to environmental changes. The stability of T3 hormone levels is considered a positive indicator of thyroid gland function stability and metabolic efficiency in birds, which supports their health and productive performance [32].

The significant improvement in the concentrations of thyroid hormones (T3 and T4) in the Shilajit supplementation treatments, whether added to drinking water or feed, compared with the first treatment (control) and the second treatment (Vitamin E supplementation), may be attributed to the presence of flavonoids in Shilajit powder [33]. Flavonoids are known antioxidants that can improve the levels of metabolic hormones [34]. Flavonoids possess a high capacity to resist oxidative stress within thyroid gland tissues, acting as effective antioxidants that reduce free radicals. This helps maintain the integrity of thyroid follicular cells and enhances their ability to secrete the hormones T3 and T4. Moreover, flavonoids stimulate the enzyme 5'-deiodinase, which is responsible for converting the hormone thyroxine (T4) into the biologically active form triiodothyronine (T3). This

T7: addition of 2 g Shilajit / kg feed.
T8: addition of 3 g Shilajit / kg feed.

conversion increases metabolic activity and improves the physiological performance of broiler chickens. Furthermore, flavonoids are believed to positively influence the hypothalamic–pituitary–thyroid axis by stimulating the secretion of thyroid-stimulating hormone (TSH), which in turn activates the thyroid gland to produce T3 and T4 more efficiently [35].

Regarding the significant improvement observed in T4 hormone concentration in the Vitamin E treatment compared with the first treatment (control), this may be attributed to the important role of Vitamin E in the metabolism of the amino acid tyrosine, which is considered a fundamental precursor in the synthesis of thyroid hormones. Vitamin E also acts as an antioxidant and contributes positively to increasing metabolic activity in the bodies of birds [36].

Conclusions and Recommendations

The addition of shilajit, whether through drinking water or feed at different levels, led to an improvement in the hematological parameters of broiler chickens, in addition to the vitamin E treatment, compared with the first treatment (control). The shilajit treatments (fourth, fifth, seventh, and eighth) showed an improvement in the concentration of total protein, while all experimental treatments recorded an increase in albumin concentration and a decrease in uric acid concentration compared with the first treatment (control). Furthermore, all experimental treatments showed an improvement in the concentrations of thyroid hormones compared with the first treatment (control).

We recommend adding Shilajit to drinking water and broiler feed at a level of 3 ml/L of drinking water and 3 g/kg of feed as a natural antioxidant throughout the rearing period to improve productive and physiological characteristics.

We also recommend using Shilajit as a natural alternative or supplement to synthetic antioxidants such as Vitamin E to improve the physiological condition of broiler chickens.

References

- [1]Bahri, S.I.S.; Ariffin, A.S. and Mohtar, S. 2019. Critical review on food security in Malaysia for broiler industry. International Journal of Academic Research in Business and Social Sciences, 9 (7), 869–876.
- [2]Ali NA-L.; Al-Nasrawi MAM.and Al-Kassie GA .2021. Investigation on the effect of adding diverse concentrations of aqueous extract of oregano leaves (*Origanum vulgare*) on physiological and immunological behaviors of broiler. Biochem. Cell. Arch., 21: 2657–2661.
- [3]Hammod AJ.; Ali NA.; Alkassar AM. and Jameel Y.J. 2018. Theeffect of partial replacement of maize by date pits on broilerperformance. J. Pure Appl. Microbiol., 12(2): 807–813.
- [4]Castro, F.L.S.; Chai, L.; Arango, J.; Owens, C.M.; Smith, P.A.; Reichelt, S.; DuBois, C. and Menconi, A. 2022. Poultry industry paradigms: connecting the dots. Journal of Applied Poultry. Res. 32:100310. 1-17.
- [5]Hamzah Merzah, L. and Abdul-Lateef Ali, N.2022. Effect of Adding Different Levels of Maca Root (*Lepidium Meyenii*) to the Diet on the Productive Performance of Broilers Exposed to Oxidative Stress. Archives of Razi Institute, 77(4), pp. 1363–1370 .
- [6]Kamgar,E.; Massoud, K. and Joanna, Z.2023. A Comprehensive Review on Shilajit: What We Know about Its Chemical Composition . Critical Reviews in Analytical Chemistry, DOI:10.1080/10408347.2023.2293963 .
- [7]Agarwal, S. P.; Khanna, R.; Karmarkar, R.; Anwer, M. K., and Khar, R. K. 2007. Shilajit: a review. Phytotherapy Research, 21(5), 401–405.
- [8]Vyas ,D. and Kumar, A. 2014. Effect of different level of Shilajit on growth performance in broilers. Veterinary Practitioner 15(1):97-98 .
- [9]Pečjak, M.; Leskovec, J.; Levart, A.; Salobir, J. and Rezar, V. 2022. Effects of dietary vitamin

E, vitamin C, selenium and their combination on carcass characteristics, oxidative stability and breast meat quality of broiler chickens exposed to cyclic heat stress. *Animals*, 12(14), 1-14.

[10]Calik, A.; Emami, N.K.; White, M.B.; Walsh, M.C.; Romero, L.F. and Dalloul, R.A. 2022. Influence of dietary vitamin E and selenium supplementation on broilers subjected to heat stress, Part I: Growth performance, body composition and intestinal nutrient transporters. *Poultry Science*, 101(6), 1-10.

[11]Layth, H.M. and Nihad, A.A.2023. Different Diets of Maca Roots (*Lepidium meyenii*) Effect Several Physiological Blood Characteristics of Broiler Chickens Under Oxidative Stress . IOP Conf. Series: Earth and Environmental Science. 1259 (2023) 012071.

[12]Duncan, D.B .1955. Multiple range multiple F-test-Biometeics.11:1-42

[13]SAS. 2012. Statistical Analysis System, User's Guide. Statistical. Version 9.1th ed. SAS. Inst. Inc. Cary. N.C. USA

[14]Feed stuffs **Ingredient Analysis Table**. 2016. Edition Prepared by Amy Batal and Nick Dale ; Sanderson Farms; University of Georgia, Athens, Ga.

[15]Ghosal, S.; Singh, S. and Kumar, Y. 2019. Shilajit in health and disease: A review. *Journal of Ethnopharmacology*, 236, 1-13.

[16]Sharma, M.; Gupta, R. and Singh, N. 2022. Immunological Benefits of Shilajit. *Immunology Research Journal*, 34(3), 150-164.

[17]Carrasco-Gallardo, C.; Guzmán, L. and Maccioni, R.B. 2012. Shilajit: A natural phytocomplex with potential therapeutic activities. *International Journal of Alzheimer's Disease*, 1-4

[18]Kasinathan, K.; Prathvi, S ; Keerthi, P.; Shetty, M.R. and Nisarga, G. C.2025. Shilajit: A comprehensive scientific review of its nature, health benefits, chemistry, and future prospects. *World Journal of Pharmaceutical and Medical Research*. 11(7), 82-89 .

[19]Sadiq, R.K.; Abrahamkhil, M.A.; Rahimi, N.; Banuree , S.Z. and Banuree, S.A.H. 2023. Effects of Dietary Supplementation of Vitamin E on Growth Performance and Immune System of Broiler Chickens. *J. World Poult. Res.*, 13(1): 120-126.

[20]Galawezh Kh. Qader, and Ihsan T.Tayeb. 2024. Influence of medicinal plants and vitamin E on productive performance and some physiological parameters of broiler chickens under heat stress. *Iraq Journal of Agricultural Sciences*. 55(6),2012-2024.

[21]Attia, Y.A.; Al-Harhi, M.A., and Hassan, S.S. 2017. Effect of dietary supplementation of vitamin E on productive performance, immunity and antioxidant status of broiler

chickens. *Animal Production Science*, 57(6), 1140–1147.

- [22]Elagib, H.A.A.; Nabiela, E.M.; Abbass, S.A. and Ginawi, T.A.N. 2012. Effect of Natural Spices on Plasma Proteins in Broiler Chicks . *Journal of Nutrition and Food Sciences* 2:152.
- [23]Igor, A.S.; Gang, X.; Mark, A.J. and Mark, T.Q. 2009. Complement-fixing Activity of Fulvic Acid from Shilajit and Other Natural Sources *Res.*; 23(3): 373–384.
- [24]Csilla, T.; Edina, S.; Blanka, G.; Veronika, G.; Veronika, O. and Oskar, N. 2024.Changes in Serum Protein Profile in Laying Hens Housed in a Cage-Free System. *Veterinary Medicine International*, Volume, Article ID 4135744, 9 pages.
- [25]Nafi, H. H. and Abdul, M. M. 2016. Effect of adding different levels of citric and acetic acids to the diet on productive traits of broiler chickens. *Al-Kufa Journal of Agricultural Sciences*, 8(1).
- [26]Al-Hasani, D.H. 2000. *Physiology of domestic poultry*. Ministry of Higher Education and Scientific Research, University of Baghdad, Dar Al-Kutub for Printing and Publishing, Baghdad.
- [27]Mayer, J. and Thomas, M. 2013. *Clinical Veterinary Advisor Birds and Exotic Pets*. ISBN 978-1-4160-3969-3. The Kenneth S. Warren Institute, Ossining, New York. DOI: <https://doi.org/10.1016/C2009-0-36486-7>
- [28]Mishraa ,T. ; Harcharan, S. Dhaliwala ; Karan, S. and Nasib, S .2019. Shilajit (Mumie): Current Status of Biochemical, Therapeutic and Clinical Advances. *Current Nutrition & Food Science*, 15, 104-120 .
- [29]Yaqoob, Z.; Batool, S.A.; Khan, A.; Hussain, R.; Raza, M.A.; Alqahtani, M.S. and Rehman, M.A.U. 2023. Characterization and medicinal applications of Karakoram shilajit; angiogenesis activity, antibacterial properties and cytotoxicity. *Materials Research Express*, 10 (10), 105403.
- [30]Al-Darraji, H. J.; Al-Hayani, W. K. and Al-Hasani, A.S. 2008. *Poultry blood physiology*. Ministry of Higher Education and Scientific Research, University of Baghdad, College of Agriculture.
- [31]El-Kholie, E.; Asmaa, G.N. and Mosaad, A. 2025. Assessing the Impact of Shilajit on Biological and Biochemical Parameters in Diabetic Rats. *Journal of home Economics, Menoufia University*, 35 (4), 111-122 .
- [32]Jessica, R. and Edward, M.D. 2023. Thyroid hormone manipulation influences development of endothermy and hatching in white leghorn chickens (*Gallus gallus*). *Journal of Thermal Biology*, Volume 114, 103582.

- [33]Aziz, S.; Sidra, K.; Habib, R.; Kh. Shakeel, G.; Muhammad, I.; Ivan, R. G. and Hidayat, H. 2017. Phytochemical Screening and Biological Studies of Shilajit (Asphaltum) .International Journal of Phytomedicine.9(1) :15-19 .
- [34]Haro, A.; López-Aliaga, I.; Lisbona, F.; Barrionuevo, M.; Alférez, M. J. and Campos, M.S. 2000. Beneficial effect of pollen and/or propolis on the metabolism of iron, calcium, phosphorus, and magnesium in rats with nutritional ferropenic anemia. Journal of agricultural and food chemistry, 48(11), 5715-5722.
- [35]Wu, J.; Jia, C.; Zhang, Z.; Hou, Z. and Cui, Y. 2024 .The relationship between dietary total flavonoids and thyroid function in u.s.adults, nhanes 2007– 2010. plos one 19(5).
- [36]Cinar, M.; Yildirim, E.; Yigit, A.A.; Yalcinkaya, I.; Duru, O.; Kisa, U. and Atmaca, N. 2014. Effects of dietary supplementation with vitamin C and vitamin E and their combination on growth performance, some biochemical parameters, and oxidative stress induced by copper toxicity in broilers. Biological Trace Element Research, 158, 186-196.