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Impact of extracts from Heamatococcus pluvialis and Magnolia officinalis, as well as vitamin E supplementation, on a number of blood biochemical indicators in laying hens

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ABSTRACT
The experiment aimed to study the Individual and combining effect of adding Heamatococcus pluvialis extract and Magnolia officinalis extract compared to vitamin E to the diet on some serum biochemical parameters of laying hens in the late phase of the laying cycle and under conditions of heat stress. At 52 weeks of age, 120 Lohman Brown laying hens were used, and the experiment continued for five periods (28 days period^{-1}) 20 weeks, from 20/3/2022 to 7/8/2022, The laying hens were randomly distributed into eight treatments, with 5 replications for one treatment and 3 hens for one replicate, The treatments included: T1 standard diet without any additives, T2 adding vitamin E to the diet at a level of 300 mg kg^{-1} feed, T3 and T4 adding Heamatococcus pluvialis extract to the diet at levels of 200 and 400 mg kg^{-1} feed respectively T5 and T6 adding Magnolia officinalis extract to the diet at the level of 200 and 400 mg kg^{-1} feed respectively and T7 and T8 adding Heamatococcus pluvialis extract mixture at a level of 100 mg kg^{-1} feed and Magnolia officinalis extract at a level of 100 mg kg^{-1} feed and 200 mg kg^{-1} feed and Magnolia officinalis extract at a level of 200 mg kg^{-1} feed respectively to the diet. The results showed that the addition of natural antioxidants (Heamatococcus pluvialis extract and Magnolia officinalis extract) and synthetic (vitamin E) to the diet of laying hens led to a significant improvement ($P\leq 0.05$) in all biochemical parameters in blood serum compared to treatment T1, and it was noted that treatment T8 (a mixture of Heamatococcus pluvialis extract and Magnolia officinalis extract) had a significant superiority ($P\leq 0.05$) in the level of total protein and globulin, and a significant decrease ($P\leq 0.05$) in the level of cholesterol, triglycerides and LDL in the blood serum of chickens. We conclude from this that adding natural antioxidants to the diet of aged laying hens which was cultured during the hot season in Iraq leads to an improvement in the blood serum's biochemical characteristics and decreases the effect of oxidative stress.

Keywords: Heamatococcus pluvialis, Magnolia officinalis, vitamin E

1. INTRODUCTION

Although the commercial laying hens reach the maximum productive performance of eggs during the peak of production, which does not last for a long time as it is noted that egg production decreases at the end of the productive peak of eggs, although the chicken still has enormous potential in producing eggs, due to the low content of eggs Ovarian precursor of yolk and premature aging of the ovaries [1,2], Oxidative stress is one of the main factors involved in premature aging of the ovaries, which occurs due to the accumulation of reactive oxygen species (ROS) in the body, Other factors affecting production, including health status, age, nutrition, breeding system, heat stress, and normal levels of free radicals maintain functions, However high levels of ROS can lead to oxidative damage to cells [3,4], In addition it was found that the ideal temperature for laying hens to perform their vital activities is 19-22 °C, Even so, it was discovered that continuing exposure to high ambient temperatures for laying hens will lead to a serious imbalance in thermal balance with the inability to lose excess body heat, resulting in heat stress that reduces from feed intake, egg quality and production; and reproductive system disruption from reduced blood supply enhanced peripheral circulation, decreased ability of the intestine to absorb nutrients, as well as the suppressive effects of the hormones prolactin and corticosterone on the adrenal gland axis, which includes the suppression of follicular growth in the ovary by the two hormones corticosterone through a decrease in the availability of vitellogenin and inhibition of gonadotrophic-pituitary-hypothalamic axis secretion including inhibition of gonadotropin-releasing hormone (GnRH) secretion from the hypothalamus and this inhibition of gonadotropin-stimulating hormone (LH and FSH) secretion from the

pituitary gland [5, 6].Therefore it has been observed recently that international companies have adopted several methods to modify the nutritional diets of poultry in line with the productive state and public health such as adding vitamin E, which is an important fat-soluble nutrient that is indispensable for its roles in animal production. When it is added to the chicken diet it protects it from The effect of oxidative stress in the body through the improvement of biochemical characteristics and indicators of oxidative stress in blood plasma [7,8], There are two main sources of vitamin E: the natural form of a-tocopherol which is widely available in plants and photosynthetic organisms and the synthetic form of all a-tocopherols which is the result of the reaction between tri methyl hydro quinone and synthetic isofitol used as a food substitute of natural vitamin E [9, 10], Numerous studies have demonstrated that the bioavailability of synthetic vitamin E is only half of that of natural vitamin E (a-tocopherol), Natural vitamin E was found to be superior to the synthetic form in aspects of antioxidant capacity and stimulation of the immune system [11, 12] .

Due to the reduction in the addition of drugs and antibiotics to avoid the precipitation of potential residues in eggs producers depend at the present time on the use of safe alternatives to improve the health of chickens and improve the continuity of egg production and resistance to oxidative stress, especially after the end of the peak of egg production for laying hens [13, 14].

The aim of adding natural antioxidants to laying chicken diets is not only to reduce oxidative stress, but it should not negatively affect the health of the consumer and be inexpensive such as adding natural Heamatococcus pluvialis extract, which is one of the pigments in the xanthophyll family its color is orange-red Belongs to carotenoids and is mainly produced by Haematococcus pluvialis, an aquatic algae that grows in freshwater, It is one of the main pigments found in some aquatic animals including trout, shrimp, wild salmon and lobster [15, 16] .

It has received a lot of attention recently for its various beneficial properties, as it has a very strong antioxidant effect that is 14, 65, 54 times or more than the effect of vitamin E, C and beta-carotene, respectively, so it has a major role in reducing the effect of free radicals in body tissues through Prevention of oxidation of cholesterol, triglycerides and proteins, and this is reflected in an improvement in the productive performance of laying hens [17-19] .

Magnolia officinalis extract is a plant polyphenol isolated from the bark of the Magnolia officinalis plant, It has gained a lot of attention nowadays because of its biological effects in reducing the effect of free radicals. Its molecular formula is $C_{18}H_{18}O_2$, It has a variety of vital functions including a powerful antioxidant and antibacterial [20,21] It is also considered an anti-inflammatory [22,23] , Researchers Deng et al. [24] showed that Magnolia officinalis extract plays a positive role in maintaining intestinal health, as it can attenuate intestinal apoptosis induced by intestinal E. coli infection in mice, protect the integrity of the intestinal mucosa from harmful oxidative stress, and maintain the balance of intestinal secretions. And its absorption, in addition to the multiple functions of Magnolia officinalis extract, it is considered to be highly safe when used [25] , Chen et al. [26] showed that the addition of Magnolia officinalis extract at a level of 200 mg kg-1of feed to the diet of laying hens at the age of 50 weeks led to an improvement in each of the productive performance, the qualitative parameters of eggs, the metabolism of hepatic fats and the function of the intestinal mucosal barrier, This study aimed to know the effect of adding natural antioxidants (Heamatococcus pluvialis extract and Magnolia officinalis extract), which are safe, non-traditional, and economically low, compared to synthetic vitamin E to the diet, and their effect on some serum biochemical parameters of laying hens.

2. MATERIALS AND METHODS

This research was carried out on a farm of laying hens raised in cages owned by the Department of Animal Production, College of Agriculture, University of Anbar, over the course of five periods (28 days period-1) and a 21-day pre-experiment period from March 20, 2022, to August 7, 2022, 120 52-week-old Lohman Brown laying hens were used in the experiment. They were divided into 8 treatments with 5 replications (3 birds per replication), with 15 chickens per treatment housed in 40 cages with the following measurements: 44 x 49 x 43 cm^2. The chickens were fed daily at eight o'clock in the morning at the rate of 110 gm of feed per bird per day, according to the diet shown in Table (1), Treatments T1 included the control treatment of basic diet without any addition, T2 adding vitamin E to the diet at the level of 300 mg kg^{-1} feed, T3 and T4 adding Heamatococcus pluvialis extract to the diet at 200 and 400 mg kg^{-1}feed, respectively, T5 and T6 adding Magnolia officinalis extract to the diet at 200 and 400 mg kg^{-1} of feed, respectively, and T7 and T8, adding a mixture of Heamatococcus pluvialis extract at a level of 100 mg kg^{-1} of feed + Magnolia officinalis extract at a level of 100 mg kg^{-1} of feed and 200 mg kg^{-1} of feed + Magnolia officinalis extract at a level of 200 mg $kg^{-1}$of feed, sequentially to the diet. Heamatococcus pluvialis extract at the level of 100 mg kg^{-1} of feed + Magnolia officinalis extract at the level of 100 mg kg^{-1} of feed and 200 mg $kg^{-1}$of feed + Magnolia officinalis extract at the level of 200 mg $kg^{-1}$of feed respectively to the diet.

The daily lighting schedule was 16 hours light and 8 hours dark per day , Drinking water was provided freely through the nipple system, A natural ventilation system was used through air vacuums, The farm was equipped with a desert cooling system.

Vitamin E (α-Tochopherol acetate) was used, manufactured at a concentration of 50% obtained from the local markets of the city of Baghdad by the United Veterinary Medicines Industry Company, a private shareholding limited company (Jordanian Yovidco), and it was added on the basis of the active substance, and 100% pure natural Heamatococcus pluvialis extract was added in a form Haematococcus pluvialis extract powder, imported from China, produced by Xi'an Changyue Phytochemistry Co.,Ltd. As for the natural magnoolol, its purity was 98%. It was added in the form of a powder extracted from the bark of the Magnolia officinalis plant imported from China and produced by Hangzhou Source Herb Bio-Tech co., LTD.

Table 1. Composition of the experimental laying hen basal diet and calculated chemical analysis.

Ingredients	**%**
Yellow corn	41
Wheat	22
Soy bean meal (44%)	23.8
Premix*	2.5
Vegetable Oil	1.2
Limestone	8.5
Salt	0.1
Di calcium phosphate	0.9
Total	100
Chemical analysis **	
ME kcal. Kg	2771
CP %	16.68
Lysine %	0.94
Met. + Cys. %	0.76
Ca %	4.11
Available Phosphor %	0.37

* The premix utilised (of Intraco/Belgian origin) has 250 mg kg-1 of methionine and 24.96% calcium. Soybean meal from Argentina, with a metabolizable energy of ME 2230 kcal and a crude protein content of 44%. kg-1.
** Chemical analysis according to [27].

At the conclusion of the fifth period, laying hens that were 72 weeks old were randomly selected to have their wing veins sampled for blood; one bird per replication (5 chickens per treatment). The test tube containing the blood samples was devoid of any materials. In order to separate the serum, which was maintained at a temperature of -20°C, the anticoagulant was then put in a centrifuge for 15 minutes at a speed of 3000 revolutions per minute. The analyses were carried out in the central laboratory of the College of Agriculture/University of Anbar, which included measuring glucose concentration [28], total protein [29], albumin protein, globulin [30], and total cholesterol [31], triglycerides [32], HDL [33] and LDL [34] , following the instructions on the kit provided by Biolabo-France manufacturer.

Serum biochemical data were analyzed statistically in one way (One way analysis), as the trend represented the effect of the eight treatments, following the General Linear Model, and using the ready-made SAS statistical program, version 10.0. 2. [35]. Significant differences between the averages were compared by Duncan, [36] multinomial test, and at the significance level of 0.05 and 0.01.

3. RESULTS AND DISCUSSION

The changes in the biochemical indicators of the blood are reflected in the health and productivity status of the laying hens, Based on this the main parameters of the blood serum of laying hens at the age of 72 weeks were studied, as shown in Table (2), as the data indicates that the addition of antioxidants significantly reduces blood glucose Laying chickens, as there was a significant increase ($P \leq 0.05$) in the blood serum glucose level in the control treatment compared to the rest of the experimental treatments which did not differ significantly between them, The high level of glucose in the blood in the control treatment compared to the rest of the experimental treatments may be due to oxidative stress, which leads to a decrease in the mass of β cells in the pancreas through apoptosis, and that this gradual decrease in the mass of β cells and their dysfunction eventually leads to Insulin deficiency and consequently high blood glucose to high levels [37-39], in addition to that the secretion of the corticosterone hormone increases when birds are exposed to various environmental stresses especially heat stress, as this hormone works to supply the body with glucose from non-carbohydrate sources especially from protein (Gluconeogenesis) [40], The decrease in the level of glucose in the treatments of addition of natural antioxidants in the diet of aged laying hens may be due to the Individual and combining role of

Heamatococcus pluvialis extract and Magnolia officinalis extract as effective antioxidants by increasing the activity of glutathione peroxidase, catalase and superoxide dismutase in the body [41,43], which contribute to increasing the effectiveness of somatic cells, which leads to enhancing the secretion of the hormone insulin, which lowers the level of blood glucose, thus reducing the effect of any condition or condition that affects the health of the bird's health, according to what studies indicated [16,44,47].

The results of the study in table (2) agreed with the findings of Rahman and Alkatan [48], who found when adding vitamin E at the level of 600 mg / kg feed to the diets of laying hens led to a significant decrease in the level of glucose in the blood serum, and also agreed with the results obtained Mohammed et al. [49] showed that the addition of industrial vitamin E at a level of 300 mg / kg feed to diets and compared it to natural antioxidants.

The results of the analyzes in the blood serum showed a significant superiority ($P \leq 0.05$) in the level of total protein of the treatment T8 (a mixture of Heamatococcus pluvialis extract 200 mg / kg feed + Magnolia officinalis extract 200 mg / kg feed) to the diet compared to the treatments T1, T4 and T5, as it was observed that the treatment The first recorded a significant decrease ($P \leq 0.05$) compared to the treatment of the addition of antioxidants. The concentration of total protein in the blood plasma is positively related to the production of eggs, as it plays a large and important role in the balance of fluid volume between blood and tissues, as it is an important carrier such as fats, carbohydrates, vitamins and minerals and hormones transport many nutritional compounds from different tissues of the body to another and it also has an important role in immunity, transmission of genetic information (DNA) formation of enzymes and hormones, and balancing blood pressure in tissues [50- 52] .

Certainly the high level of total protein in blood serum (within normal limits) is considered a good indicator of health status and evidence of egg production because the vast majority of egg components pass through the blood to the ovary and are bound to protein [53]. Among the results shown in Table (2) we notice a significant decrease ($P \leq 0.05$) in favor of T1 (control treatment) in the level of total protein in the blood serum, it may be due to the occurrence of oxidative stress within the body that leads to an increase in protein oxidation processes in the blood plasma and this causes protein loss and decrease its blood level [54] .

Table (2) shows the level of albumin in the serum of laying hens as a significant ($P \leq 0.05$) was observed in T3, T5 and T6, and these treatments did not differ with T2 and T4 compared to T1, T7 and T8, as there was a significant decrease in them ($P \leq 0.05$). in serum albumin level. As for the level of globulin, the results showed a significant superiority ($P \leq 0.05$) in favor of T8 (a mixture Heamatococcus pluvialis extract 200 mg kg^{-1} feed + Magnolia officinalis extract 200 mg kg^{-1} feed) over all treatments, which did not differ significantly with T7 (a mixture of Heamatococcus pluvialis extract 100 mg kg^{-1} feed + Magnolia officinalis extract 100 mg kg^{-1}feed) compared to the rest of the experimental treatments.

The significant decrease ($P \leq 0.05$) obtained in the control treatment compared to the antioxidants addition treatments in the concentration of total protein, globulin and albumin in blood plasma may be due to the accumulation of lipid peroxidation products in liver cells, and this leads to a decrease in the function of the liver in protein synthesis and Damage to the membranes and liver cells as the ability to synthesize protein in the liver is an important indicator of liver activity [55], In addition, the increase in protein oxidation in the blood plasma is associated with an increase in the production of free radicals resulting from stress, which leads to the loss of protein and a decrease in its level in the blood plasma [56], From the results we note that vitamin E and natural antioxidants have a role in improving the level of globulin in the blood serum by stimulating humoral immunity which means an increase in the number of B lymphocytes and this in turn leads to an increase in immunoglobulins and it is known that immune stimulation causes an increase and division of cells T-type lymphocytes, which in turn increase the production of lymphokines giving the latter to the body the greatest possible benefit from the nutrients inside when digested and metabolized, thus increasing the process of biosynthesis protein in the body [56-58].

Also an increase in the concentration of protein in the blood serum gives an indication of an increase in the metabolic rate and an increase in the activity of the hormone thyroxine produced by the thyroid gland, which in turn works to increase the stimulation of protein synthesis [59,60], the reason for the high concentration of total protein, albumin, and globulin in the blood serum of laying hens to the treatments of adding natural antioxidants may be due to the Individual and combining role of Heamatococcus pluvialis extract and Magnolia officinalis extract in maintaining the structure of the cell membrane by limiting the effect of free radicals and preventing the occurrence of oxidation without causing any damage to it, thus preserving the cell membrane. The level of amino acids and raising the level of proteins as well as reducing the effect of corticosterone being a protein damaging hormone and stimulating it to produce lymphocytes responsible for building some immune proteins such as globulins and albumins [26,61,62], Also it may be due to the role and effectiveness of Heamatococcus pluvialis extract as an antioxidant when it is absorbed and accumulated in the tissues of the body this leads to protecting the liver from the effect of stress and increasing its activity in making protein as well as decreasing its catabolism processes [63-65], Albumin is one of the main proteins responsible for stabilizing the body and maintaining its natural balance the reason for the superiority in the level of globulin in favour of the addition

treatments may be attributed to the role of Heamatococcus pluvialis extract by increasing the production of lymphocytes that work to reduce stress factors and raise the immune response of the body [58,66,67], Grimes et al. [68] also showed that Heamatococcus pluvialis extract has the ability to activate the bone marrow to produce B lymphocytes that raise the level of immune performance against diseases, Jagruthi et al.[61] found that Heamatococcus pluvialis extract increases the levels of both total protein and globulin. And albumin in blood serum, as globulin is considered one of the important blood proteins to maintain a healthy immune system for the body, and albumin is important for maintaining osmotic pressure by preventing the exit of blood fluids from blood vessels into the tissues of the body in addition to linking vital compounds with blood and transporting them to different parts of the body [69], Also among Zhu et al. [70] when adding Heamatococcus pluvialis extract to the diet of laying hens at the age of 60 weeks resulted in a significant superiority ($P \leq 0.05$) in the level of immunoglobulins, as they explained that the mechanism of action of Heamatococcus pluvialis extract in improving immune function may be due to its role in promoting nutrition and absorption of nutrients and providing The nutrients required for the production of immune protein, this study agreed with the findings of Lin et al. [43] that the addition of Magnolia officinalis extract to the duck diet at a level of 200 and 300 mg / kg feed to the duck diet led to an increase in the level of total protein and globulin in the blood plasma compared to the treatment the control.

Table 2. Impact of extracts from Heamatococcus pluvialis and Magnolia officinalis, as well as vitamin E supplementation, on some biochemical properties of serum of laying hens.

Treatments	Glucose (mg $100ml^{-1}$)	Total protein (g L^{-1})	Albumin (g L^{-1})	Globulin (g L^{-1})
T1	230.00±15.56 a	62.66±2.02 d	38.33±2.33 d	27.00±3.00 dc
T2	183.33 ± 5.54 b	82.33±0.66 abc	51.33±1.20 ab	31.00±1.52 dc
T3	186.00 ± 8.14 b	87.66±1.85 ab	54.00±1.73 a	33.66±2.72 bc
T4	175.00 ± 3.05 b	79.00±3.46 c	52.00±1.52 ab	27.00±2.08 dc
T5	182.66 ± 3.71 b	80.66±3.84 bc	56.33±2.33 a	24.33±1.85 d
T6	167.33 ± 1.76 b	82.00±1.15 abc	56.66±3.52 a	25.33±3.33 d
T7	178.33 ± 6.88 b	85.33±1.20 abc	46.33±1.66 bc	39.00±2.64 ab
T8	169.66 ± 5.23 b	88.33±1.45 a	43.33±0.88 bc	45.00±2.00 a

* N.S.: Not significant at the significant level ($P \leq 0.05$). A significant difference between the treatments at a significant level ($P \leq 0.05$) is shown by the different letters within a single column (a, b, c). T1: Control , T2:Vitamin E(300 mg kg^{-1} feed) , T3: Heamatococcus pluvialis extract (200 mg kg^{-1}feed), T4: Heamatococcus pluvialis extract (400 mg kg^{-1}feed), T5: Magnolia officinalis extract(200 mg kg^{-1}feed), T6: Magnolia officinalis extract(400 mg kg^{-1}feed), T7: mixture Heamatococcus pluvialis extract + Magnolia officinalis extract (100 mg kg^{-1}+ 100 mg kg^{-1}feed), T8: mixture Heamatococcus pluvialis extract + Magnolia officinalis extract (200 mg kg^{-1}+ 200 mg kg^{-1}feed).

Table (3) shows the results of the lipid profile of blood serum, The results showed that adding different levels of antioxidants to the diets of laying hens had an effect in reducing the level of triglycerides in the blood plasma. The decrease was significant ($P \leq 0.05$) T8 (mixture Heamatococcus pluvialis extract 200 mg kg^{-1} + Magnolia officinalis extract 200 mg kg^{-1}feed) compared to the treatments T1, T3, T5 and T6, which recorded a significant increase ($P \leq 0.05$) and did not differ significantly with the treatments T2, T4 and T7, also the results show that all treatments recorded a significant decrease ($P \leq 0.05$) in the serum cholesterol level of laying hens compared with the control treatment, which showed a significant increase ($P \leq 0.05$),The blood content of triglycerides and cholesterol is one of the important indicators that characterize the production of eggs in laying hens as their accumulation in the blood in high proportions as in the control treatment can be considered as evidence of a decrease in the efficiency of the use of nutrients especially fats as a result of oxidative stress at the same time the sharp decrease can be considered as an indicator this may lead to a defect in the general functional activity of the body [71,72].

The reason for the decrease in the level of serum cholesterol in the second treatment (adding synthetic vitamin E 300 mg kg^{-1} feed) may be that the level of vitamin E in the diet increases the rate of storage (deposition) of vitamin E in the body as it is stored in adipose tissue with fats and this It enhances its role in protecting cellular membranes from oxidative damage by maintaining the optional permeability in the cell wall because vitamin E works in a complex with unsaturated fatty acids finally this prevents cholesterol and fats from leaking out of the cellular membranes because they are sites for their leakage which result from reactions Oxidation of fats in the liver plasma and egg yolk and this works to reduce the level of cholesterol and fats as shown in Table (3) When laying hens are exposed to stress vitamin E contains a hydroxyl group through which it gives a hydrogen atom

to the free radical of fat and thus the oxidation of fats is inhibited, and this is also reflected in providing protection from oxidation of low-density lipoproteins (LDL), and it follows that vitamin E works On reducing the level of lipids (hypolipdemic) by preventing the oxidation of LDL and increasing the level of HDL in blood plasma [73,74] .

Table 2. Impact of extracts from Heamatococcus pluvialis and Magnolia officinalis, as well as vitamin E supplementation, on the lipid profile of blood serum of laying hens.

Treatments	Triglycerides (mg $100ml^{-1}$)	Cholesterol (mg $100ml^{-1}$)	HDL (mg $100ml^{-1}$)	LDL (mg $100ml^{-1}$)
T1	645.66±8.98 a	393.00±14.57 a	43.66±2.60 d	219.33±18.55 a
T2	601.66±14.25 ab	221.33±11.55 c	60.66±3.52 ab	40.33±11.86 c
T3	625.00±24.02 a	254.00±6.08 bc	49.33±1.45 dc	79.33±4.63 bc
T4	556.66±48.44 ab	251.66±17.03 bc	62.66±0.88 a	77.33±25.16 bc
T5	643.66±72.07 a	260.66±17.07 bc	55.00±1.73 bc	77.00±3.21 bc
T6	629.00±10.78 a	271.00 ± 7.93 b	55.33±0.88 bc	90.00±8.73 b
T7	587.33±27.47 ab	261.66±14.76 bc	53.00±2.08 c	91.00±12.28 b
T8	500.33±46.41 b	272.00±5.50 b	55.00±2.30 bc	117.00±7.09 b

* N.S.: Not significant at the significant level (P≤0.05). A significant difference between the treatments at a significant level (P≤0.05) is shown by the different letters within a single column (a, b, c). T1: Control , T2:Vitamin E(300 mg kg^{-1}feed) , T3: Heamatococcus pluvialis extract (200 mg kg^{-1}feed), T4: Heamatococcus pluvialis extract (400 mg kg^{-1}feed), T5: Magnolia officinalis extract(200 mg kg^{-1}feed), T6: Magnolia officinalis extract(400 mg kg^{-1}feed), T7: mixture Heamatococcus pluvialis extract + Magnolia officinalis extract (100 mg kg^{-1}+ 100 mg kg^{-1}feed), T8: mixture Heamatococcus pluvialis extract + Magnolia officinalis extract (200 mg kg^{-1}+ 200 mg kg^{-1}feed).

The reason for the significant decrease (P≤0.05) in the level of serum cholesterol in favor of the addition of natural antioxidants may be due to the Individual and combining role of adding Heamatococcus pluvialis extract and magnolia officinalis extract to laying hens diets as many studies indicated that Heamatococcus pluvialis extract is a natural carotenoid that has a very strong antioxidant effect, It is 14, 65, and 54 times or more greater than the effect of vitamin E, C, and beta-carotene respectively so it has a significant role in reducing the effect of free radicals in body tissues through the prevention of oxidation of cholesterol, triglycerides, and proteins, and thus improving the health of the body [17,19], And the decrease in the level of cholesterol due to the treatment of the addition of magnolia officinalis extract which is a vegetable polyphenol has gained more attention due to its vital effects in reducing the effect of free radicals, Since polyphenols are important for promoting the expression of the cholesterol enzyme 7 alpha hydroxylase, which regulates bile synthesis and cholesterol balance, they also prevent the activity of hydroxyl 3-methyl-glutaryl-CoA, an enzyme that restricts the body's ability to synthesise cholesterol. [23,75,76], also between Yuan et al.[77] that magnolia officinalis extract has an effect in reducing the activity of Acyl-CoA acyltransferase (ACAT), which is responsible for the esterification of cholesterol, and thus is the reason for lowering the level of cholesterol in the blood.

The results of the statistical analysis in Table (3) showed that there was a significant superiority (P≤ 0.05) in the level of high density lipoproteins (HDL) in favor of the T4 treatment (Heamatococcus pluvialis extract 400 mg kg-1feed), which did not differ significantly with the second treatment compared to the rest of the experimental treatments, As for low density lipoprotein (LDL) the control treatment recorded a significant (P≤0.05) superiority compared to the rest of the experimental treatments, in which a significant decrease (P≤0.05) was observed in the level of low density lipoprotein in blood serum , The low density lipoprotein molecule (LDL) is formed when the triglyceride molecule is completely cleaved by the enzyme lipoprotein lipase in the tissues outside the liver, The molecule from various somatic cells for the purpose of using it in the process of cellular construction and the manufacture of products that include fat in its construction and composition [50], and that the significant increase (P≤0.05) in the level of LDL that occurred in favor of the control treatment compared to the rest of the treatments of adding antibiotics Oxidation may be due to the occurrence of oxidation of LDL, which leads to its accumulation in the blood at a high level, This is a result of its attack by free radicals and thus its loss of functional effectiveness and this leads to a change in its shape,and finally a decrease in the ability of the liver cell receptors to recognize and take it and this leads to the occurrence of Decrease in the rate of metabolic reactions related to triglycerides cholesterol and phospholipids, and this leads to an increase in their concentration and accumulation in the blood as a result of Decreased LDL particle clearance rates from blood and elevated LDL levels [78,79], This may be the reason for the decrease in the blood serum cholesterol level in the treatments of adding natural antioxidants to the addition of Heamatococcus pluvialis extract and Magnolia

officinalis extract to the diets of laying hens, so it is necessary to add different types of antioxidants to the diet to reduce the effects of free radicals in poultry [80], the results agreed With what Iwamoto et al.[81], showed when consuming Heamatococcus pluvialis extract from marine sources, it prevents the oxidation of low-density lipoprotein in the blood and thus prevents its level from rising above the normal level in the body.

The reason for the ability of Magnolia officinalis extract additions to lower cholesterol and triglyceride levels in blood serum may be due to the fact that polyphenols have high antioxidant activity by suppressing the activity of free radicals especially the negative superoxide radical and the hydroxyl radical [82] and activating body antioxidants (glutathione peroxidase and glutathione) as well as reducing the level of malondialdehyde (MDA) products also its ability to restrict oxidation catalysts (bound iron) and reduce lipolysis and thus works to protect low-density lipoproteins from oxidation [83], Wang et al.[84] showed that polyphenols reduce serum LDL levels due to increased excretion of cholesterol with waste products, Endogenous cholesterol is produced by the liver and transported to extrahepatic tissues by low-density lipoprotein[85]. At the same time, high density lipoprotein transports cholesterol from peripheral tissues to the liver before being excreted via bile, Gao et al.[86] The results agreed with the findings of [20] When laying hens at 50 weeks of age were fed Heamatococcus pluvialis extract at a dose of 100 mg kg-1feed, their blood plasma levels of total cholesterol and LDL significantly decreased in comparison to the other experimental treatments, as agreed the results are consistent with the findings of Mohammed et al.[49] as they showed that the addition of natural and synthetic antioxidants to the diet of laying hens led to a significant decrease ($P \leq 0.05$) in the level of triglycerides, The blood plasma of laying chickens exposed to heat stress included low density lipoproteins and total cholesterol.

4. CONCLUSION

The addition of different levels of Heamatococcus pluvialis extract or Magnolia officinalis extract as natural antioxidants in both forms individually or in combination, to the diet of aged laying hens during the hot season in Iraq leads to an improvement in some biochemical parameters of blood serum and reduces the effect of oxidative stress in an almost better way when compared with an antioxidant Synthetic (vitamin E).

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