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Article

Effect of Dietary Supplementation with Dried Olive Leaf Powder and L-Carnitine on Hematological and Biochemical Blood Parameters of Broiler Chickens (Ross 308)

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Abstract: Natural feed additives have received increasing attention in poultry nutrition due to their potential role in improving physiological status and reducing metabolic disorders in broiler chickens. Olive leaves are rich in phenolic compounds and antioxidants, while L-carnitine plays an essential role in fatty acid metabolism and energy production. This study was conducted at the broiler chicken farm of the Animal Production Unit, College of Medicinal and Industrial Plants, University of Kirkuk, Iraq, from 9 September 2024 to 13 October 2024. A total of 180 broiler chicks (Ross 308) were used in the experiment. At 7 days of age, the birds were randomly allocated to six experimental treatments with three replicates each: T1 (control), T2 (1 g/kg dried olive leaf powder), T3 (2 g/kg dried olive leaf powder), T4 (500 mg/kg L-carnitine), T5 (1 g/kg dried olive leaf powder + 500 mg/kg L-carnitine), and T6 (2 g/kg dried olive leaf powder + 500 mg/kg L-carnitine). Birds were fed a starter diet until 21 days of age followed by a grower diet until 35 days, with feed and water provided ad libitum. The results showed that dietary supplementation with dried olive leaf powder and L-carnitine, either individually or in combination, significantly improved several hematological and biochemical blood parameters compared with the control treatment. The supplemented treatments showed increased concentrations of total protein, albumin, and high-density lipoprotein (HDL), along with significant reductions in total cholesterol, triglycerides, low-density lipoprotein (LDL), and very low-density lipoprotein (VLDL). In addition, the activities of liver enzymes aspartate aminotransferase (AST) and alanine aminotransferase (ALT) were reduced, indicating improved liver function and metabolic status. Moreover, lymphocyte percentage and the heterophil/lymphocyte (H/L) ratio were not negatively affected. Dietary supplementation with dried olive leaf powder and L-carnitine can improve biochemical blood parameters and metabolic health in broiler chickens (Ross 308), suggesting that these additives may serve as effective natural feed supplements in broiler production systems.

Keywords: olive leaf powder, L-carnitine, broiler chickens, hematological parameters, blood biochemistry.

Introduction

The production of broilers has become an essential aspect of the poultry sector and the animal production sector due to the significant role played in the attainment of food security [1], [2]. The broiler sector is one of the fastest-growing sectors in the agricultural sector, and it plays an essential role in the attainment of food security through the provision of protein, energy, and nutrients through the production of broilers within the shortest time [3], [4].

The synthetic antioxidant is often used in the production of poultry products through the addition of the antioxidant to the poultry diet, leading to the improvement of the quality of the carcass and the productivity of the broilers [5]. The synthetic antioxidant, however, is reported to have low efficacy and is associated with potential side effects, which may limit the production of healthy meat products from poultry that is safe for human consumption [6]. The medicinal plants and herbs have been added to the poultry diet due to their ability to act as antioxidants [7], [8].

Researchers have shown that the olive leaves are not less significant than the olive fruits, playing an important role in the health of human beings and animals [9]. The olive leaves have several biological properties, including antioxidant, antimicrobial, antibacterial, anti-inflammatory, and anticoagulant activities, as well as the ability to lower blood pressure [10]. The olive leaves are composed of various bioactive compounds such as oleanolic acid, olein, oleasterol, oleuropein, olivine, and olestranol, as well as amino acids such as glutamate, proline, serine, mannitol, and aspartate [11], [12]. Among the components of the olive leaves, the most significant antioxidant is the oleuropein, which is responsible for the improvement of the health of animals [13]. The olive leaves have been used to treat several health conditions such as stress, atherosclerosis, gout, rheumatism, diabetes, and fever in human beings [14], [13].

L-carnitine is an amino acid or a vitamin-like substance that is synthesized in the body from the amino acids lysine and methionine [15], [16]. The chemical structure of L-carnitine is $C_7H_{15}NO_3$ [17], [18]. The substance is vital in the energy metabolism or the metabolic processes in the human body. The substance is vital in the metabolism of fatty acids in the human body. It is responsible for the transport of long-chain fatty acids into the mitochondria for the production of energy in the form of adenosine triphosphate (ATP) in the mitochondria [19], [20]. In broiler nutrition, the use of L-carnitine in the diet has been shown to improve the nutritional status of broilers, mainly due to the sparring action of the two amino acids lysine and methionine, which are the precursors of the substance. This will enhance the efficiency of the utilization of energy in broilers [21]. Additionally, L-carnitine decreases the availability of lipids for oxidation by transporting fatty acids to the mitochondria and increasing ATP synthesis [22]. Moreover, owing to its antioxidant properties, L-carnitine enhances the activities of antioxidant enzymes, glutathione peroxidase, and superoxide dismutase in the plasma [23].

Thus, the objective of the current study was to investigate the effect of dietary supplementation with varying levels of dried olive leaf powder, alone or in combination with L-carnitine, on some biochemical blood parameters of broiler chickens of the Ross 308 variety.

Materials and Methods

This study was conducted at the broiler chicken farm of the Animal Production Unit, College of Medicinal and Industrial Plants, University of Kirkuk, Iraq, during the period from 9/9/2024 to 13/10/2024, to investigate the effect of dietary supplementation with dried olive leaf powder and L-carnitine on the biochemical blood parameters of broiler chickens (Ross 308).

A total of 180 broiler chicks (Ross 308) were used in this experiment. At 7 days of age, the chicks were randomly distributed into six experimental treatments, each consisting of three replicates, as follows:

T1: Control treatment
T2: Control + 1 g/kg feed dried olive leaf powder
T3: Control + 2 g/kg feed dried olive leaf powder
T4: Control + 500 mg/kg feed L-carnitine
T5: Control + 1 g/kg dried olive leaf powder + 500 mg/kg L-carnitine

T6: Control + 2 g/kg dried olive leaf powder + 500 mg/kg L-carnitine

The chicks were given the control diet for the first 8 days, after which the experimental diets were given. Birds were given a starter diet from 7 to 21 days of age, with a composition of 23.59% crude protein and 3000 kcal/kg metabolizable energy, followed by a grower diet from 21 to 35 days of age, with a composition of 21.7% crude protein and 3081 kcal/kg metabolizable energy. Ingredients used in the feed were obtained from Noor Poultry Feed Factory in Kirkuk. Chicks were obtained in the early hours of the morning from a hatchery. At 8 days of age, the chicks were weighed and randomly distributed in cages. Water was given using inverted drinkers with a capacity of 4 L, while feed was given in plastic trays in the first week, after which it was replaced with suspended cylindrical feeders with a diameter of 45 cm and adjusted to the level of the back of the birds.

The feed and water were given ad libitum throughout the experiment. The temperature was maintained at 33°C in the first week, after which it was gradually reduced to 23-24°C in the third week of the birds' age. Lighting was maintained at 23 hours light and 1 hour darkness in the first week to acclimatize the chicks to darkness, after which it was reduced to 20 hours light and 4 hours darkness. Relative humidity was maintained within the recommended range based on the broiler management guide, while the ventilation fans were kept at low speed in the early days and gradually increased as the birds grew. Blood sampling was conducted from the brachial (wing) vein using a 5-ml syringe with a 25-gauge needle, based on the method used by Al-Darraji et al. At the end of the experiment (35 days of age), blood sampling was conducted from two birds per replicate (i.e., six birds per treatment). The blood samples were collected in tubes with anticoagulant for hematological studies and in tubes without anticoagulant for serum biochemical studies. The blood samples were kept in the refrigerator for 12 hours before centrifugation at 3000 rpm for 15 minutes to separate the serum from the cellular components. The serum was collected using a micropipette and kept in the freezer before the laboratory tests were conducted.

The data collected were analyzed statistically using the Completely Randomized Design (CRD) to test the significance of the effects of the treatments given to the birds on the traits studied. Duncan's [24] Multiple Range Test was used to compare the significant differences between the treatment means, and the statistical analysis was conducted using the Statistical Analysis System (SAS) software [25].

Table 1. Ingredient composition (%) and calculated chemical composition of experimental diets Ingredient composition.

Feed ingredient	Starter (7–21 d)	Grower (21–35 d)
Yellow corn (%)	17.31	20.12
Wheat (%)	39.29	43.68
Soybean meal (49%)	34.75	29.15
Sodium chloride (%)	0.30	0.22
Sunflower oil (%)	4.00	4.00
Limestone (%)	1.00	0.60
Dicalcium phosphate (%)	2.66	1.32
Vitamin–mineral premix (a)	0.10	0.10
Methionine (%)	0.27	0.25
Lysine (%)	0.32	0.32
Choline chloride (60%)	—	0.24

Total	100	100
Calculated chemical composition		
Nutrient	**Starter**	**Grower**
Metabolizable energy (kcal/kg diet)	3000.53	3097.76
Crude protein (%)	23.67	21.75
Methionine (%)	0.59	0.55
Lysine (%)	1.44	1.31

(a) One kilogram of vitamin–mineral premix provides: Vitamin A (12,000 IU), Vitamin D3 (2,500 IU), Vitamin E (200 mg), Vitamin K3 (20 mg), Vitamin B1 (20 mg), Vitamin B2 (50 mg), Vitamin B6 (30 mg), Vitamin B12 (150 μg), folic acid (10 mg), niacin (300 mg), calcium (8%), manganese (400 mg), zinc (150 mg), iron (53 mg), copper (43 mg), and choline (40 mg). **(b)** Calculated according to the chemical composition of feed ingredients based on **NRC** [26].

Results

The findings of the statistical analysis presented in Table 2 revealed the impact of the experimental treatments on the hematological values such as WBC counts ($\times 10^3/\mu L$), heterophil percentage, lymphocyte percentage, and the heterophil to lymphocyte ratio in broilers (Ross 308).

The findings revealed that there are significant differences among the experimental treatments with regard to WBC counts; however, numerical differences were observed in the WBC counts of the treatments that received dried olive leaf powder and L-carnitine, as well as the control treatment. In the case of the heterophil percentage, it was revealed that the value of the treatment T1 was significantly higher ($P \leq 0.05$) than the values of treatments T2, T3, T4, T5, and T6; however, numerical differences were observed in the values of the treatments that received dried olive leaf powder and L-carnitine.

In the case of the lymphocyte percentage, no significant differences among the treatments that received dried olive leaf powder and L-carnitine were observed; however, the values of the treatments that received dried olive leaf powder and L-carnitine did not show significant differences in the H/L ratio. The findings revealed that the use of dried olive leaf powder and L-carnitine did not induce physiological stress in broilers; however, the absence of negative effects in the values of the treatments that received dried olive leaf powder and L-carnitine with regard to the lymphocyte percentage and the H/L ratio revealed that the use of the dietary additives did not induce adverse physiological responses.

Table 2. Effect of dietary supplementation with dried olive leaf powder and L-carnitine on hematological parameters of broiler chickens (Ross 308) (Mean ± SE).

Treatment	WBC ($\times 10^3/\mu L$)	Heterophils (%)	Lymphocytes (%)	H/L ratio
T1	6.19 ± 0.17 ab	31.66 ± 2.96 a	62.00 ± 2.30 a	0.57 ± 0.09 a
T2	6.27 ± 0.42 ab	25.66 ± 1.76 ab	63.66 ± 0.33 a	0.42 ± 0.13 a
T3	6.74 ± 0.23 a	24.00 ± 1.73 b	61.33 ± 1.76 a	0.40 ± 0.09 a
T4	5.69 ± 0.22 b	26.66 ± 0.88 ab	61.00 ± 1.52 a	0.44 ± 0.08 a
T5	6.06 ± 0.39 ab	30.33 ± 1.76 ab	62.66 ± 1.20 a	0.56 ± 0.16 a
T6	5.69 ± 0.29 b	28.66 ± 1.76 ab	62.66 ± 1.20 a	0.45 ± 0.03 a

Treatments were as follows: T1 = control (0 g/kg), T2 = control + 1 g/kg dried olive leaf powder, T3 = control + 2 g/kg dried olive leaf powder, T4 = control + 500 mg/kg L-carnitine, T5 = control + 1 g/kg dried olive leaf powder + 500 mg/kg L-carnitine, and T6 = control + 2 g/kg dried olive leaf powder + 500 mg/kg L-carnitine. Different letters within the same column indicate significant differences among treatments at ($P \leq 0.05$).

The results shown in Table 3 indicate the effect of the experimental treatments on some biochemical blood parameters, such as globulin, total protein, and albumin. The results indicated that the concentration of total protein in the blood plasma was significantly increased in treatments T5 and T6, compared to treatments T1, T2, T3, and T4. Moreover, treatments T2, T3, and T4 had higher values of total protein than treatment T1, but no differences existed between treatments T2, T3, and T4. Regarding the albumin concentration, treatments T4, T5, and T6 had significantly higher values than treatments T1, T2, and T3. Moreover, treatments T2 and T3 had significantly higher values of albumin than the control treatment, T1. However, the globulin concentration was not significantly affected.

Table 3. Effect of dietary supplementation with dried olive leaf powder and L-carnitine on biochemical blood parameters of broiler chickens (Ross 308) (Mean ± SE).

Treatment	Globulin (mg/dl)	Total protein (mg/dl)	Albumin (mg/dl)
T1	4.10 ± 0.17 a	6.34 ± 0.60 c	2.23 ± 0.29 c
T2	4.31 ± 0.11 a	8.25 ± 0.57 b	3.26 ± 0.28 b
T3	4.13 ± 0.05 a	8.36 ± 0.66 b	3.56 ± 0.27 ab
T4	3.95 ± 0.08 a	9.36 ± 0.84 b	4.07 ± 0.04 a
T5	4.09 ± 0.10 a	11.40 ± 0.34 a	3.97 ± 0.07 a
T6	3.97 ± 0.14 a	12.62 ± 0.40 a	3.98 ± 0.09 a

The results obtained in the study presented in Table 4 showed the effect of the dietary supplements with dried olive leaf powder and L-carnitine on the levels of some blood lipid fractions, such as cholesterol, triglyceride, high-density lipoprotein, low-density lipoprotein, and very low-density lipoprotein, in broiler chickens at the age of 35 days. The results showed a significant decrease in the serum level of cholesterol in the serum of broiler chickens in the following treatment groups, T2, T3, T4, T5, and T6, compared with the control treatment group, T1. In addition, the results showed a higher level of reduction in the level of cholesterol in the serum of broiler chickens in the following treatment groups, T5 and T6, compared with the other treatment groups, T1, T2, T3, and T4.

Regarding the effect of the supplements with dried olive leaf powder and L-carnitine on the level of triglyceride in the serum of broiler chickens, the results showed a significant decrease in the level of triglyceride in the serum of broiler chickens in the following treatment groups, T5 and T6, compared with the control treatment group, T1. In addition, the results showed a significant decrease in the level of triglyceride in the serum of broiler chickens in the following treatment groups, T2, T3, and T4, compared with the control treatment group, T1.

Regarding the effect of the supplements with dried olive leaf powder and L-carnitine on the level of high-density lipoprotein in the serum of broiler chickens, the results showed that the highest level of high-density lipoprotein was recorded in the following treatment group, T6, with a significant difference compared with the control treatment group, T1. In addition, the results showed that there was no significant difference between the following treatment groups, T5 and T6, and the results also showed that the levels of high-density lipoprotein in the serum of broiler chickens in the following treatment groups, T2, T3, and T4, were significantly higher compared with the control treatment group, T1.

Regarding the effect of the supplements with dried olive leaf powder and L-carnitine on the level of low-density lipoprotein in the serum of broiler chickens, the results showed a significant decrease in the level of low-density lipoprotein in the serum of broiler chickens in the following treatment groups, T2, T3, T4, T5, and T6, compared with the

Table 4. Effect of dietary supplementation with dried olive leaf powder and L-carnitine on blood lipid profile of broiler chickens (Ross 308) (Mean ± SE).

Treatment	Cholesterol (mg/dl)	Triglycerides (mg/dl)	HDL (mg/dl)	LDL (mg/dl)	VLDL (mg/dl)
T1	90.94 ± 1.74 a	81.76 ± 0.95 a	27.20 ± 1.73 d	47.18 ± 2.91 a	16.22 ± 0.24 a
T2	85.83 ± 1.18 b	77.23 ± 0.56 b	31.14 ± 1.09 cd	33.24 ± 0.32 b	15.44 ± 0.91 ab
T3	78.59 ± 1.20 c	73.91 ± 1.68 b	32.30 ± 1.86 c	29.76 ± 1.80 b	14.78 ± 0.92 abc
T4	80.91 ± 0.85 c	69.71 ± 0.35 c	36.37 ± 1.05 b	30.26 ± 1.63 b	13.94 ± 0.07 bc
T5	76.84 ± 1.39 cd	64.15 ± 1.10 d	40.26 ± 0.70 ab	28.39 ± 0.77 bc	12.83 ± 0.42 c
T6	73.82 ± 1.34 d	62.81 ± 1.39 d	40.76 ± 0.76 a	23.59 ± 1.23 c	15.22 ± 0.54 ab

Normally, under physiological states, the enzymes aspartate aminotransferase (AST) and alanine aminotransferase (ALT) are found in the cells of the body's tissues. However, when tissues are damaged, the enzymes are released into the blood, and their concentration increases, indicating tissue damage resulting from diseases, injuries, or metabolic disorders [24].

The results in Table 5 indicate differences ($P \leq 0.05$) among the treatments supplemented with dried olive leaf powder and L-carnitine compared with the control treatment for AST activity. Treatments T5 and T6 had significantly lower AST levels compared with T1, T2, T3, and T4. On the other hand, T2, T3, and T4 were not significantly different from each other, but they were significantly different from the control treatment (T1).

The results also indicate a significant decrease ($P \leq 0.05$) in ALT activity for all the supplemented treatments compared with the control treatment (T1). Treatments T3, T4, and T6 had lower ALT activity compared with the control treatment (T1). These results indicate improved liver functions and reduced liver stress in broiler chickens following supplementation with olive leaf powder and L-carnitine.

Table 5. Effect of dietary supplementation with dried olive leaf powder and L-carnitine on liver enzyme activity of broiler chickens (Ross 308) (Mean ± SE).

Treatment	AST (U/L)	ALT (U/L)
T1	21.83 ± 1.02 a	28.86 ± 2.46 a
T2	19.90 ± 1.30 ab	24.45 ± 1.65 ab
T3	18.82 ± 0.92 ab	17.68 ± 1.21 c
T4	20.04 ± 0.19 ab	17.93 ± 0.89 c
T5	17.98 ± 1.16 b	20.90 ± 1.70 bc
T6	17.78 ± 0.92 b	17.19 ± 1.75 c

Discussion

The increase in the concentration of total protein in the blood serum, as observed in this study, may be due to the antioxidant effect of olive leaves, which enhances liver protection and improves liver functions. Olive leaves contain several bioactive compounds, such as those responsible for stimulating protein synthesis, regenerating, and producing liver cells [27]. Additionally, the increase in the concentration of protein in the blood serum of broiler chickens supplemented with L-carnitine may be associated with the synthesis of certain amino acids, as it is considered a precursor for several amino acids and vitamins [16], [28]. Similar findings were observed by Parizadian et al. who reported an increase in albumin concentration in the blood serum of quails supplemented with L-carnitine compared with the control group [29]. Furthermore, Jalali et al. indicated that the increase in the concentration of total protein in the blood serum of broiler chickens supplemented with L-carnitine may be associated with the protein-sparing effect of L-carnitine, as it reduces the utilization of certain amino acids such as lysine and methionine for the synthesis of carnitine [30].

Several researchers have proven the physiological benefits of olive leaves on poultry. Ahmed et al. showed that dietary administration of an extract of olive leaves to two-week-old broiler chickens after fasting reduced their serum glucose levels by 7-38% [31]. Al-Nuaimi showed that dietary administration of crushed olive leaves at 1g/kg feed to broiler chickens reduced their serum glucose levels [32]. The hypoglycemic effect of olive leaf powder can be attributed to its high antioxidant activity, which protects pancreatic cells from oxidative damage, boosts antioxidant defense systems in the pancreas, and regulates insulin secretion to maintain normal blood glucose levels. In addition, it is possible that olive leaf powder induces insulin secretion from pancreatic β-cells, which stimulates glucose uptake into storage sites such as the liver and muscles, where it is stored as glycogen, thereby lowering blood glucose levels above normal [33].

The considerable reduction in serum cholesterol levels recorded in this study upon administration of olive leaf powder supplement may be attributed to two major factors. Firstly, it is postulated that the high levels of dietary fiber contained in olive leaves increase the binding time of cholesterol and bile acids, thereby inhibiting their absorption into the blood and instead increasing their excretion through feces. Consequently, this leads to an increase in the conversion of blood cholesterol into new bile acids, thereby lowering blood cholesterol levels [34], [35]. Secondly, it is postulated that the active components present in olive leaves, such as unsaturated fatty acids, oleanolic acid, vitamins, and other bioactive compounds, have been known to protect low-density lipoproteins from oxidative changes [36]. In addition, olive leaves have been known to have other active components, such as natural pigments, which have been reported to have high levels of polyphenol and tannin compounds, which have been known to have antioxidant properties and antimicrobial activity [37]–[38]–[39]. This is supported by findings by Fayed et al. who recorded a considerable reduction in serum cholesterol and triglyceride levels in broiler chickens fed on diet supplemented with olive leaf powder [40].

The beneficial effects of L-carnitine on lipid metabolism have also been reported. Jalali et al. suggested that dietary supplementation of L-carnitine increases the activity of the enzyme carnitine acetyltransferase, which increases the transport of acetyl-CoA from the mitochondria to the cytosol [41]. Acetyl-CoA is believed to be one of the major precursors for the synthesis of cholesterol. Moreover, Lien and Horng suggested that dietary supplementation of L-carnitine increases the activity of the enzyme carnitine palmitoyltransferase, which is responsible for the oxidation of fatty acids [42]. As a result, the production of very low-density lipoproteins is decreased in the liver. An increase in fatty acid oxidation may also decrease the accumulation of triglycerides in the adipose tissues. Moreover, Xu et al. suggested that supplementation of 250 mg L-carnitine/kg feed into the diet of laying hens decreased the levels of triglycerides in the serum due to an increase in the mitochondrial fatty acid oxidation [43]. In addition, the reduction of low-density lipoproteins may also be due to the inhibition of the activity of the enzyme β-hydroxy-β-methylglutaryl-CoA reductase, which is the major enzyme for the synthesis of cholesterol, due to the supplementation of L-carnitine.

With regards to liver enzyme activity, the decrease in AST and ALT levels observed in the current study indicates improved liver functions and reduced liver stress in broiler chickens supplemented with olive leaf powder and L-carnitine. Under normal physiological states, AST and ALT are intracellular enzymes found in many tissues, although their levels increase in the bloodstream when tissues are damaged.

Conclusion

The results of this study show that dietary supplements of dried olive leaf powder and L-carnitine, alone or in combination, improve several blood parameters of broiler chickens. The improvement of blood parameters is indicated by an increase in total protein and HDL levels, as well as a decrease in cholesterol, triglycerides, LDL, and liver enzyme activity levels in broiler chickens supplemented with olive leaf powder and L-carnitine. This indicates that the combination of olive leaf powder and L-carnitine may improve the metabolic and liver functions of broiler chickens, and as such, it may be recommended as a natural feed supplement for improving the physiological and metabolic functions of broiler chickens in modern poultry production systems.

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