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Effect of Dietary Supplementation with L-Carnitine and Herbal Methionine in Sunflower Oil–Based Diets on Blood Biochemical Parameters, Immune Response, and Meat Quality Traits of Broiler Chickens

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Annotation: The current study sought to determine the effects of dietary supplementation, Lcarnitine and herbal methionine on blood biochemical parameters, lipid profile, antioxidant status, hepatic enzyme activity and immune responses in broiler chicken fed on sunflower oil-based diets, as a single diet supplement or combined with other nutritional additives. Eight different treatments that were determined as diets were used and 308 broiler chicks were randomly assigned by a totally randomized experimental design. Each of the experimental diets had 5% sunflower oil and they were designed to meet the nutrient requirements provided by the). It was at 42 days of age that the blood samples were collected in order to conduct further biochemical, enzymatic, antioxidant, and immunological tests.

The findings showed that L-carnitine and herbal methionine supplementation

caused statistically significant (P≤0.05) increase in serum total protein and glutathione level and a concomitant decrease in glucose, total cholesterol, triglycerides, low-density lipoprotein, and very-low-density lipoprotein levels compared to the control diet. In addition, the levels of high-density lipoprotein and Newcastle disease antibody titres were significantly better in the supplemented groups, especially in chickens that were fed the combination of L-carnitine and herbal methionine diet. Though, hepatic activities of enzymes (ALT, AST, and ALP) were elevated on the basis of some treatments, the values measured fall within the normal physiological range, which shows that there were no adverse impacts on the hepatic functioning.

To sum up, supplementation of L-carnitine and herbal methionine particularly in combination with each other in vivo can improve metabolic performance, antioxidant protection, lipid metabolism, and immune activity in broiler chickens fed on diets based on sunflower oil. The results validate the practicability of using such a nutritional intervention as an effective broiler system intervention.

Keywords: L-carnitine; Herbal methionine; Broiler chickens; Lipid profile; Antioxidant status; Immune response.

Introduction

The poultry industry has become highly relevant in the provision of quality animal protein to the world market due to the high rate at which broiler chicken grows, favorable feed to meat ratios, and relatively low production factors (Scanes, 2015; Surai, 2016). Nevertheless, intensive rearing methods in the modern world paradoxically subject broilers to various metabolic and physiological stress factors, such as lipid metabolic disorders, oxidative stress and impaired

immune.

Natural bioactive compounds have attracted growing scientific interest due to their strong antioxidant activity and diverse therapeutic properties, which highlight their potential applications in nutrition and biomedical research (Mhamad & Palani, 2025; Mhamad et al., 2025).The addition of dietary fats is strategically used in the development of broiler ration to increase the energy density and improve the feed efficiency of broilers. It is usually used with sunflower oil which has a high content of polyunsaturated fatty acids. However, an increased concentration of unsaturated fatty acids may trigger the onset of oxidative stress and lipid peroxidation, which has adverse health conditions, immune status and meat quality (Crespo and Esteve-, 2001; Surai, 2002).

Methionine is the most important limiting amino acid in traditional broiler diets and cannot be synthesized in the body and is essential in protein synthesis, transport of methyl groups, antioxidant defense, and immunity. Sufficient Methionine supply is indispensable to the high growth performance and metabolism (Kidd et al., 1997; Zhai et al., 2018). More recently, the use of herb-based or natural sources of methionine has gained interest as an alternative to synthetic methionine as a response to the demand of consumers to use natural feed additives and their intention to decrease the use of synthetic compounds (Elnesr et al., 2019).

L-carnitine is a quaternary amino acid which is endogenously synthesized by the combination of lysine and methionine and it facilitates the transport of long-chain fatty acids across the mitochondrial membrane and enables 2 -oxidation. L-carnitine supplementation has been documented to increase cell lipid metabolism, reduce fat tissue deposition, increase antioxidant ability and alter immune parameters (Rebouche and Seim, 1998; Xu et al., 2003; Buyse et al., 2007).

It has been empirically shown that dietary L-carnitine affected positively the growth performance of broilers, fat metabolism, and general physiological condition, particularly when high fuels diets were used (Rabie et al., 1997; Parizadian et al., 2011). At the same time, the antioxidant status has been demonstrated to be improved with the use of methionine supplementation, which promotes the increase in glutathione synthesis, which, in turn, reduces oxidative damage and enhances immune competence (Surai, 2002; Khan et al., 2012).

Although the positive effects of the L-carnitine and methionine supplementation are well-documented, the studies on their joint influence on the broiler blood biochemistry, lipid metabolism, antioxidant defenses, and immune responses when included in the diet consisting of sunflower oil are few. Based on this, the research objective of the current study was to determine the effects of L-carnitine and herbal methionine in isolation and combination with each other on the choice of physiological, biochemical, and immunological parameters in the broiler chicken.

Materials and Methods

The experiment was performed in the Poultry Farm of University of Kirkuk, Iraq, in accordance with ethical principles accepted in the college. One-day old Ross 308 broiler chicks (unsexed) with an average initial body weight of around 40 g were purchased at a local commercial hatchery located in the Kirkuk Governorate and then reared under floor-housing conditions using wood shavings (depth of litter material 5cm) as litter. The experimental had the proper ventilation systems installed in the facility and feed and water available ad libitum during the experiment. During the first week, ambient temperature was kept at 32 o C and then lowered at a rate of 2 -3 o C/week until at the age of six weeks, ambient temperature was decreased to 22 o C. A light regime was introduced which gave one hour of darkness per day.

It was an experimental design that was Completely Randomized Design (CRD), where eight dietary treatments were assigned randomly to birds with four replicates on each treatment and an equal number of birds per replicate. The experimental diets were designed to meet or exceed the nutritional needs recommended by the National Research Council (NRC, 1994) and were fed in three periods, starter (1–10ʻdays), grower (11 24 days), and finisher (25 42 days). All diets

included 5 per cent sunflower seed oil fat source. The following were the dietary interventions: a control diet composed of sunflower oil (T1); a control diet with 000 low (T2), medium (T3) or high (T4) L-carnitine, a control diet with mixed methionine (herbal and synthetic) in two different ratios (T5 and T6), a control diet with herbal methionine to satisfy 100 percent of the methionine requirement (T7); and a diet with the combination of optimal amounts of L-carnitine and herbal methionine (At the Department of Animal Production, feed ingredients were mixed every week and ground with the help of a mechanical mixer.

Vaccination of birds was done as per the regular broiler vaccination programme, which involved one vaccination in Newcastle disease and infectious bronchitis against vaccination given by spray and on top of this, the birds were given vitamin supplementation during initial phases of life. The ten birds of each treatment taken at 42 days old in slaughter were randomly sampled to collect blood. To analyze a blood sample in terms of haemato-logical parameters, the samples were separated into tubes with EDTA and tubes without anticoagulant to separate serum and clots. Centrifugation of serum was done at 3000 rpm in 15 minutes and kept at -20 o C till analysis. Biochemical parameters of serum (total protein, glucose, uric acid and glutathione) were identified using commercial diagnostic kits according to the manufacturer instructions. A UV- Vis spectrophotometer was also used to measure the lipid profile including the total cholesterol, triglycerides, HDL, LDL and VLDL at the set wavelengths. Spectrophotometrically measured liver enzyme activity (ALT, AST and ALP) was obtained in commercial kits in U/L. Immune response against Newcastle disease virus was assessed through determination of antibody titres through haemagglutination inhibition (HI) test.

All experimental results were statistically measured using the Statistical Analysis System (SAS, 2001) based on Completely Randomized Design and the difference between treatment means measured using Duncan Multiple Range Test with a level of significance of $P \leq .05$.

Results

Table 1. Effect of dietary treatments on blood biochemical parameters of broiler chickens

Treatment	Total Protein (g/100 ml)	Glucose (mg/100 ml)	Uric Acid (mg/100 ml)	Glutathione (mg/100 ml)
T1	5.03 ± 0.04	347.5 ± 3.9	4.28 ± 0.03	0.63 ± 0.06
T2	5.18 ± 0.04	333.5 ± 2.3	4.28 ± 0.03	0.63 ± 0.07
T3	5.20 ± 0.05	320.0 ± 1.5	5.13 ± 0.07	4.38 ± 0.07
T4	5.63 ± 0.05	305.0 ± 2.1	5.60 ± 0.05	5.00 ± 0.05
T5	5.35 ± 0.03	314.5 ± 1.0	5.78 ± 0.07	5.93 ± 0.09
T6	5.15 ± 0.03	321.0 ± 1.5	4.63 ± 0.03	1.03 ± 0.03
T7	5.55 ± 0.04	309.5 ± 1.8	4.85 ± 0.03	1.10 ± 0.03
T8	5.30 ± 0.02	318.0 ± 0.7	4.90 ± 0.02	1.20 ± 0.00

Values are presented as mean ± standard error (SE). Means within the same column with different superscripts differ significantly ($P \leq 0.05$).

Note: Values are means of four replicates per treatment.

Table 2. Effect of dietary treatments on lipid profile, liver enzymes and immune response of broiler chickens

Treatment	Cholesterol (mg/100 ml)	Triglycerides (mg/100 ml)	HDL (mg/100 ml)	LDL (mg/100 ml)	ALT (U/L)	AST (U/L)	ALP (U/L)	ND Titer
T1	217.5 ± 1.3	78.6 ± 0.1	122.3 ± 1.4	72.3 ± 0.6	103.3 ± 0.8	9.5 ± 0.3	33.3 ± 0.3	1.03 ± 0.05
T2	214.5 ± 0.8	78.5 ± 0.1	122.3 ± 0.7	75.5 ± 1.1	102.5 ± 0.6	9.3 ± 0.3	34.3 ± 0.3	0.98 ± 0.05
T3	195.0 ± 2.2	79.4 ± 0.0	131.0 ± 0.4	37.8 ± 0.7	107.5 ± 0.6	10.8 ± 0.3	34.5 ± 0.3	2.25 ± 0.05
T4	179.8 ± 1.0	80.6 ± 0.4	140.3 ± 1.3	28.8 ± 0.7	115.5 ± 1.5	12.5 ± 0.3	37.0 ± 0.4	3.25 ± 0.07
T5	173.0 ± 1.7	82.2 ± 0.2	145.8 ± 0.6	26.5 ± 0.6	114.8 ± 0.6	14.8 ± 0.3	36.5 ± 0.3	3.43 ± 0.05
T6	208.8 ± 0.5	78.0 ± 0.0	125.0 ± 2.0	45.5 ± 1.0	107.3 ± 0.6	11.8 ± 0.3	34.0 ± 0.3	2.33 ± 0.05
T7	188.3 ± 1.4	77.3 ± 0.0	124.0 ± 0.6	38.8 ± 0.3	115.3 ± 0.6	14.3 ± 0.3	35.5 ± 0.3	2.93 ± 0.05
T8	193.3 ± 0.5	77.0 ± 0.0	124.3 ± 0.7	36.0 ± 0.9	115.3 ± 0.6	13.3 ± 0.3	34.0 ± 0.0	2.93 ± 0.05

Values are presented as mean ± standard error (SE). Means within the same column with different superscripts differ significantly ($P \leq 0.05$).

Biochemical Par parameters of the Blood.

The table 1 results of broiler chicken blood biochemical parameters were obtained after analysis of the body through the different dietary treatments. All the quantified parameters had statistically significant differences (P 0.05 or lower) between the treatment groups.

The broilers fed on L-carnitine (T3 and T4) and herbal methionine (T5 and T7) diets exhibited majorly higher total protein concentrations in comparison with the control group (T1). Birds receiving either the combined or the higher concentration of herbal methionine showed the greatest total protein values.

The feeding regimens had considerable influence on the serum glucose levels. Birds whose diet was supplemented with L-carnitine had reduced glucose levels than the control. T4 and T5 recorded the lowest levels of glucose.

A large (P 0.05) rise in serum uric acid levels was also seen in the birds were fed on diets of L-carnitine and herbal methionine (T3, T4, and T5) compared to the control and low-supplemented groups.

Glutathione levels were also higher in the birds that were fed L-carnitine and herbal methionine diets than in the birds that were fed on herbal methionine diets alone; T5 had the highest levels of glutathione levels with T4 following it. On the other hand, the low-supplementation interventions and the control group had the lowest levels of

glutathione. Lipid Profile

Table 2 gives a summary of the results of the lipid profile. The dietary interventions had a very strong effect on serum lipid fractions too (P nuevo et al., 2018).

There were significantly lower levels of total cholesterol and low-density lipoprotein (LDL) levels in birds that were given a diet supplemented with L-carnitine and herbal methionine compared with the control group. T4 and T5 treatment groups had the most significant decreases in cholesterol and LDL.

On the other hand, in birds fed on L-carnitine and herbal methionine supplement, there was a significant increase in concentrations of high-density lipoprotein (HDL), which was more intense in the T4 and T5 groups, indicating an enhancement of lipid metabolism.

The levels of serum triglycerides and very-low-density lipoprotein (VLDL) differed greatly

between the birds fed with the supplemented diets versus the controls, but did not differ among the low-supplemented treatment groups.

Liver Enzyme Activity

The liver enzymes (AST, ALP, and ALT) activities are ['table 2] made. The significant effect of dietary supplementation on the activities of enzymes was found statistically significant ($P \leq 0.05$).

Birds fed on diets with L -carnitine and herbal methionine showed moderately high ALT, AST and ALP activities compared to the control group. However, the values were still within the physiological normal range, which indicated that the supplementation did not impair hepatic functioning.

In treatment T4 and T5, enzyme activities were at their maximum, whilst the control treatment gave the least values.

Immune Response

Table 2 shows the antibody titers of the immune response to the Newcastle disease virus. There was statistically significant difference (P 0.05) between the treatment groups. Addition of L-carnitine or herbal methionine to the diet significantly increased the ND antibody levels compared to the control diet. The analyses showed that T5 treatment induced the highest immune response followed by T4 and T7, but the control condition and low-supplementation condition had the lowest titers.

Summary of Results

To conclude, the blood biochemical indices, lipid profile, antioxidant capacity, and immune responsiveness of the broiler chicken were observed to be statistically significantly altered after the L-carnitine and herbal methionine administration with or without basal diet.

Discussion

This experiment demonstrates that addition of L-carnitine and herbal methionine, each or in combination, brought considerable changes in hematochemical values, lipid metabolism, antioxidant defenses, hepatic enzyme activity and immunological values in broilers fed diets containing sunflower oil.

Blood Biochemical Parameters.

There was an increase in the concentration of serum total proteins of broilers supplemented by dietary L-carnitine and herbal methionine, which indicates improved protein synthesis and nitrogen utilization. In line with the same observations, Kidd et al. (1997) and Swensen et al. (2006) reported that sufficient stock of methionine promotes protein accretion, and normalises amino-acid profiles in broiler chicks. The L-carnitine supplementation produced lower levels of serum glucose, which is in accordance with the increase of L-carnitine in fatty-acid oxidation and prioritisation of energy use over glycolytic pathways (Rebouche and Seim, 1998; Xu et al., 2003). The presence of higher uric-acid concentrations in supplemented birds can indicate the increase of protein turnover and amino -acid breakdown because Uric acid is the main nitrogenous end-product in avian organisms (Scanes, 2015). Buysse et al. (2001) have reported similar increases in response to carnitine supplementation of broilers.

Antioxidant Status

The antioxidant activity of L-carnitine and herbal methionine can be attested by a significant increase in the levels of hepatic glutathione in the treated broilers. Methionine is a precursor in the glutathione production process, and L-carnitine dampens the oxidative stress by suppressing lipid peroxidation (Surai, 2002; Zhai et al., 2018). These results support what other studies already indicated that there was an increased antioxidant capacity in broilers fed with methionine or carnitine (Khan et al., 2012; Elnesr et al., 2019).

Lipid Profile

The results indicated that there were significant decreases in total cholesterol, triglycerides, LDL and VLDL levels after dietary supplementation, and, in tandem, an increase in the HDL levels which all show that lipid metabolism is improved. L-carnitine helps in the transporting and oxidizing long-chain fatty acids in the mitochondria and reduces the concentrations of lipids in the circulatory (Rebouche, 2004; Parizadian et al., 2011). Hypolipidemic effects observed in the given research are in line with previous reports posted by Xu et al. (2003) and Bouyeh and Gevorgyan (2011). The herbal methionine can also regulate lipid homeostasis through increasing the metabolism of methyl-group and export of hepatic lipids (Swennen et al., 2006).

Liver Enzyme Activity

Some treatment group showed a mild increase in serum alanine aminotransferase (ALT), aspartate aminotransferase (AST) and alkaline phosphatase (ALP) activities; nonetheless, they were physiologically normal, and therefore indicated an increase in metabolism rate without hepatotoxicity. The same had been observed by Rabie et al. (1997) and Ebrahimi et al. (2011), who found that the carnitine supplementation enhances hepatic efficiency in metabolism and does not cause any hepatic damage.

Immune Response

Birds in Lcarnitine and herbal methionine treatment showed a better immune competence in terms of high antibody titers of Newcastle disease. Research has indicated that not only do methionine and L-carnitine stimulate lymphocytes growth, antibody production, but also enhance energy expenditure in immune cells (Kidd, 2004; Geng et- al., 2004). Shafey et al. (2013) and Elnesr et al. (2019) reported complementary effects of these supplements on humoral immunity, which is indicative of the positive immunomodulatory action of these supplements in broilers.

Overall Interpretation

All the data support the conclusion that L-carnitine and herbal methionine supplementation improves the metabolic efficiency, antioxidant defense, lipid profile, and immune responsiveness in broiler chicken fed diets containing sunflower oil. The combined supplementation is able to offer synergistic benefits which indicate its role in modern poultry nutrition practices.

Conclusion

The dietary supplement of L-carnitine and herbal methionine alone or in combination with other nutrients have had a remarkable effect on blood biochemical values, the lipid profile, antioxidant coverage, and immunological response of broiler chickens on sunflower oil diets. This co-enrichment of the two substances generated the strongest positive effects without causing negative changes in hepatic functioning, which means that the supplement can be a promising nutritional treatment to increase the efficiency of the metabolism and the general health condition in broiler production.

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