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The Biological Impact of Co-Treatment with Coenzyme Q10 and Zinc Methionine on Some Biochemical Parameters in Awassi Ewes

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ABSTRACT

This study was conducted in the Department of Animal Production, College of Agriculture, and University of Tikrit, to study the impact of treatment with Coenzyme Q10 and Zinc Methionine on some blood biochemical parameters in Awassi lambs. A total of 20 Awassi lambs, aged between 4 and 4.5 months, with an average weight of 21.71 ± 0.34 kg, were utilized and randomly assigned to five treatment groups. The first group received distilled water, while the second and third groups were administered Coenzyme Q10 at doses of 25 and 50 mg per 5 ml of water per animal, respectively. The fourth and fifth groups received a combination of Coenzyme Q10 and Zinc Methionine at doses of (25 mg of Q10/ 5 ml of water/animal + 100 mg of zinc/ animal) and (50 mg of Q10/5 ml of water/animal + 100 mg of zinc/animal), respectively. The results of this study revealed significant ($P \leq 0.05$) increases in the levels of total proteins and albumin in the blood of animals in the fifth, fourth, and third treatment groups compared to the first and second groups. Moreover, a significant increase in globulin levels was observed in the fifth and third treatment groups compared to the other groups 90 days after treatment. Additionally, there was a significant ($P \leq 0.05$) decrease in the total cholesterol level in the blood serum of animals in the fifth treatment group compared to the other groups. Furthermore, there was a significant ($P \leq 0.05$) reduction in the levels of triglycerides and very low-density lipoprotein cholesterol, as well as a significant reduction in atherogenic index in the treated animals compared to the control group. Additionally, there was a significant ($P \leq 0.05$) decrease in low-density lipoprotein cholesterol levels in the blood serum of animals in the fifth and fourth treatment groups compared to the control group, while high-density lipoprotein cholesterol levels showed a significant increase in the fifth and fourth treatment groups compared to the control group after 90 days of treatment.

KEYWORDS: Coenzyme Q10, Zinc Methionine, Awassi lambs, blood biochemical parameters, protein levels, cholesterol, triglycerides, lipoprotein cholesterol, atherogenic index.

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INTRODUCTION

Sheep are among the most important livestock animals in Iraq and a primary source of meat production. Meat is a significant source of animal protein, and there is an increasing demand for it due to growing nutritional awareness among consumers (Amin, 2014). Awassi sheep are one of the important and distinguished breeds known for their good meat quality, not to mention possessing a high capability to adapt to harsh environmental and nutritional conditions (Merchen et al., 1992). Animal productivity is influenced by nutritional status. Therefore, the provided animal feed should be diverse and supplemented with non-traditional additives to enhance its nutritional value and emphasize its vital role in improving productivity, as well as enhancing the health and immunity of the animals (Steen et al., 2008; Khan et al., 2010).

Studies have focused on adding certain antioxidants and mineral elements to animal diets due to their role in protecting the body's cells from free radical damage. Additionally, they contribute to maintaining animal health and enhancing their reproductive and productive performance (Hassan et al., 2017; Balani et al., 2018; El-Nagar & Wafa, 2021). Antioxidants are considered one of the most important means of preventing the generation of free radicals resulting from various biological activities in the body or slowing down their formation. They play a defensive role against the destructive activity of free radicals (Videla et al., 2004; Law et al., 2018). One of the antioxidants used is the enzyme coenzyme Q10,

also known as ubiquinone, which is naturally present in both higher and lower organisms. It is a fat-soluble antioxidant and the only one that can be synthesized within the body (Littarru et al., 2017). Moreover, it plays a vital role in energy production as it is one of the electron and proton carriers in the oxidative phosphorylation processes within the mitochondria of the cells (Shukla and Dubey, 2018). Coenzyme Q10 also serves to protect cellular membranes and lipid proteins in the plasma from lipid peroxidation. Furthermore, it plays a role in regenerating other antioxidants, such as vitamin E and vitamin C (Gvozdjáková et al., 2015).

The biological availability of certain essential minerals is often limited in animal diets and may not fulfill the animal's nutritional requirements (Princewill et al., 2015). Trace minerals present in animal feed are not readily accessible to the animal due to their interactions with each other and binding with various chemical compounds such as phytates, oxalates, and tannins. These interactions hinder mineral absorption, resulting in trace element deficiencies within the body (Hummel et al., 2020).

Due to repeated farming, grazing, deforestation, and related activities, most soils have become deficient in essential minerals, subsequently leading to deficiencies in forage crops (Datt and Chhabra, 2005). Studies have shown that adding trace minerals to the diet in appropriate quantities participates in nutritional metabolism, growth, and reproduction, and helps in scavenging free radicals resulting from oxidative stress by participating in enzymatic activities or acting as enzyme cofactors. Additionally, they enhance immune system efficiency (Yatoo et al., 2013).

Zinc is considered one of the essential minerals that the body continuously requires since it is one of the most commonly deficient minerals in the diet. Unlike other minerals, there is no reserve storage for zinc in the body (Praharaj et al., 2021). It plays a crucial role in numerous and diverse metalloenzymes, serving as a vital cofactor for many enzymatic reactions (Hou et al., 2021).

Zinc plays a significant role in promoting growth, immunity, cell division, protein synthesis, maintaining the health and integrity of epithelial tissues, acting as a stimulant for antioxidants, and contributing to hormone secretion (Krishnaiah et al., 2019). Zinc poisoning in field animals is very rare due to the tolerance of these species to high doses of this mineral in their diet (McDowell, 1992; NRC, 1996). Based on the above, this study aimed to investigate the impact of treatment with the co-enzyme Q10 and zinc methionine on some biochemical properties in the blood of Awassi sheep.

MATERIALS AND METHODS

The experiment was conducted at the animal field of the College of Agriculture, Department of Animal Production, University of Tikrit, from March 8, 2023, to June 5, 2023. In this study, 20 local Awassi sheep, aged between 4 and 4.5 months, with an average weight of 21.71 ± 0.34 kg, were utilized.

These animals were divided into five treatment groups. The first group received distilled water, while the second and third groups were administered the coenzyme Q10 at doses of 25 mg and 50 mg/ 5 ml of water/ animal, respectively. The fourth and fifth groups received a combination of the coenzyme Q10 and zinc methionine consisting of (25 mg of Q10 + 100 mg of zinc/ 5 ml of water/ animal), and a combination of (50 mg of Q10 + 100 mg of zinc/ 5 ml of water/ animal), respectively.

The experimental animals underwent a preparatory period of 14 days during which their health was carefully monitored to prevent potential health issues. The animals were provided with concentrated fodder consisting of 21% wheat bran, 50% crushed barley, 25% crushed maize, 13% soybean meal, 10% bran, 1% calcium carbonate (limestone), and 1% table salt. The crude protein content in the diet was 14.4125%, and the energy content was 2873.5 kcal/kg. The feed was provided collectively for each treatment group, equivalent to 3% of their live weight, divided into two daily meals.

The feed quantity was adjusted every two weeks based on weight changes, with access to a freely available coarse feed (straw). Clean water and mineral salt blocks were placed in each pen for all groups.

Blood samples were collected at regular intervals every 45 days at 7:30 AM after fasting the animals for 12 hours. The blood was drawn from the jugular vein in the neck region using a sterile 10 ml syringe. The collected blood was transferred into clean and sterilized plastic tubes and allowed to clot for one hour at room temperature.

Subsequently, the tubes were placed in a refrigerator at 4°C at a 45-degree angle for 24 hours. They were then centrifuged at 3000 rpm for 20 minutes to separate the blood serum from the other components. The serum was stored in sealed tubes in a freezer at -20°C until the biochemical tests were conducted.

BLOOD TESTS

1. Total Protein Concentration Determination

The total protein concentration in the blood serum was estimated using commercial kits from the French company BIOLABO. This was done by analyzing the samples with a spectrophotometer at a wavelength of 546 nm (Burtis et al., 2012).

To calculate the total protein quantity, the following equation was applied:

Total Protein Level (g/100 ml of blood) = Sample Absorbance / Standard Solution Absorbance × 6

2. Albumin Concentration Determination

The concentration of albumin in the blood serum was determined using specialized commercial kits from the French company BIOLABO, following the method described

by Doumas et al. in 1971. To measure the albumin level, the following equation was employed:

Albumin (g/100 ml) = Sample Absorbance / Standard Solution Absorbance × 5

3. Globulin Concentration Estimation

To calculate the globulin level in the blood serum, the following equation was applied, following the method described by Bishop et al. in 2000.

Globulin Concentration (g/100 ml of Blood) = Total Protein Concentration (g/100 ml of Blood) - Albumin Concentration (g/100 ml of Blood)

4. Determination of Total Cholesterol Concentration

The concentration of total cholesterol in the serum was determined using a commercial kit from the French company BIOLABO. The serum samples were examined with a spectrophotometer at a wavelength of 500 nm. To measure the cholesterol level in the serum, the following equation was applied:

Cholesterol Concentration (mg/dL) = Sample Absorbance / Standard Solution Absorbance × 200 (Standard Concentration).

5. Determination of Triglyceride Concentration

The concentration of triglycerides in the serum was determined using commercial kits manufactured by the French company BIOLABO. Serum samples were examined with a spectrophotometer at a wavelength of 500 nm. To measure the triglyceride concentration in the serum, the following equation was applied:

Triglyceride Concentration (mg/dL) = Sample Absorbance / Standard Solution Absorbance × 200 (Standard Concentration)

6. Estimation of High-Density Lipoprotein Cholesterol (HDL-C)

The concentration of high-density lipoprotein cholesterol (HDL-C) in the serum was estimated using commercial kits manufactured by the French company BIOLABO. Serum samples were examined with a spectrophotometer at a wavelength of 500 nm. To measure the concentration of high-density lipoprotein cholesterol, the following equation was applied:

HDL-C Concentration (mg/dL) = Sample Absorbance / Standard Solution Absorbance × 100 (Standard Concentration).

7. Low-Density Lipoprotein Cholesterol (LDL-C) Estimation

The concentration of low-density lipoprotein cholesterol (LDL-C) in serum was estimated using the Friedewald equation, as proposed by Friedewald et al. (1972):

LDL-C Concentration (mg/dL) = Total Cholesterol - (HDL-C + (Triglycerides / 5)).

8. Very Low-Density Lipoprotein Cholesterol (VLDL-C) Estimation

The concentration of very low-density lipoprotein cholesterol (VLDL-C) in serum was calculated following the method proposed by Friedewald et al. (1972):

VLDL-C Concentration (mg/dL) = Triglycerides / 5

9. Atherogenic Index

The atherogenic index, indicating the risk of atherogenesis, was calculated as follows:

Atherogenic Index = Triglycerides / High-Density Lipoprotein Cholesterol

Statistical Analysis

The statistical analysis was conducted using a Complete Randomized Design (CRD) with a one-way direction. To assess the significance of differences between treatments, Duncan's multiple range test (Duncan, 1955) was employed. Statistical analysis was performed using the SAS software (SAS, 2012).

The data was analyzed using the following mathematical model:

Yij = μ + Ti + eij

Where:

- Yij = Observation value j for treatment i.
- μ = The overall mean of the studied trait.
- Ti = The effect of treatment i, where i = 1 (Control), 2(Second) , 3(Third),4 (Fourth), and 5 (Fifth).
- eij = Experimental error, which follows a normal independent distribution with a mean of zero and equal variance with a value of e2σ.

RESULTS AND DISCUSSION

1. The Effect of Treatment with the Enzyme Co-factor Q10 and Zinc Methionine on the Serum Blood protein level in Awassi Sheep.

The results in Table (1) indicate the absence of a statistically significant effect of treatment with the co-enzyme Q10 and zinc methionine on the total protein level in the serum of Awassi sheep between the different treatments after 45 days of treatment (the first duration). However, after 90 days of treatment (the second duration), significant differences were observed ($P \leq 0.05$). Animals in the third and fifth treatments showed a significant increase ($P \leq 0.05$) in the total protein level in the serum compared to the other treatments. Additionally, animals in the fourth treatment exhibited a statistically significant advantage ($P \leq 0.05$) in this trait compared to the first (control) and second treatments.

Table 1: Effect of Enzyme Co-treatment with Q10 and Zinc Methionine on the Serum Protein Levels in Awassi Lambs.

Traits	Period	Treatments					significance
		First (T1)	Second (T2)	Third (T3)	Fourth (T4)	Fifth (T5)	
Total Protein gm/100ml	First (after 45 days)	6.24 ± 0.38 a	6.55 ± 0.25 a	6.56 ± 0.20 a	6.40 ± 0.06 a	6.54 ± 0.16 a	N.S
	Second (after 90 days).	5.33 ± 0.12 c	5.40 ± 0.05 c	7.24 ± 0.39 a	6.50 ± 0.19 b	7.28 ± 0.22 a	*
Albumin Gm/100ml	First (after 45 days).	3.43 ± 0.16 a	3.87 ± 0.06 a	3.68 ± 0.13 a	3.78 ± 0.22 a	3.58 ± 0.13 a	N.S
	Second (after 90 days).	2.95 ± 0.05 b	2.87 ± 0.15 b	3.87 ± 0.38 a	3.72 ± 0.28 a	3.75 ± 0.10 a	*
Globulin gm/100 ml	First (after 45 days).	2.81 ± 0.37 a	2.69 ± 0.20 a	2.88 ± 0.27 a	2.63 ± 0.21 a	2.97 ± 0.16 a	N.S
	Second (after 90 days)	2.38 ± 0.11 b	2.53 ± 0.11 b	3.38 ± 0.10 a	2.78 ± 0.20 b	3.53 ± 0.12 a	*

The values represent means ± standard error. N.S. indicates no significant differences ($P \geq 0.05$). (*) denotes statistically significant differences ($P \leq 0.05$). T1 = Control, T2 = Enzyme co-treatment with Q10 at 25 mg/animal, T3 = Enzyme co-treatment with Q10 at 50 mg/animal, T4 = Enzyme co-treatment with Q10 at 25 mg/animal + 150 mg/animal Zinc Methionine, T5 = Enzyme co-treatment with Q10 at 50 mg/animal + 150 mg/animal Zinc Methionine.

To assess the biological impact of treatment with the enzyme co-factor Q10 and zinc methionine on the serum albumin level, no statistically significant differences were observed during the first period of treatment. However, during the second period (90 days), significant differences were noted among the five treatments. The third, fourth, and fifth treatments showed a significant increase ($P \leq 0.05$) in comparison to the first and second treatments. It's worth noting that the serum globulin level remained unaffected by the different treatments after 45 days of treatment (the first duration), but during the second duration (after 90 days of treatment), a significant increase ($P \leq 0.05$) was recorded in the blood of animals in the third and fifth treatments compared to the first, second and fourth treatments.

The improvement observed in the total protein, albumin, and globulin levels in the treated animals compared to the control group can be attributed to the role of the co-enzyme Q10 in enhancing liver function and enhancing the immune status of the treated animals. These results are consistent with the findings of Mohamed et al. (2017), who observed a significant increase in the levels of total protein, albumin, and globulin in ewes treated with 5 and 10 mg/kg of nano zinc in their feed along with their lambs 15 days after giving birth compared to the control group.

Additionally, Al-Zubaedi et al. (2021) indicated a significant increase in the levels of total protein, albumin, and globulin in the blood of goats exposed to zinc deficiency after being administered 25 and 75 mg of zinc once a week for 10 weeks.

Abo Elhaded et al. (2021) reported that treating Rahmani ewes with different zinc sources at a dose of 50 mg/kg of feed during the last two months of pregnancy and for two months after childbirth led to a significant improvement in the total protein and globulin levels during the pregnancy and postpartum stages, while the albumin level remained unaffected in all stages compared to the control group.

Kumar et al. (2021) highlighted a significant increase in globulin levels in calves treated with zinc supplements at 40 and 60 mg/kg of feed for 90 days compared to the control group.

Abdelgayed et al. (2022) recorded a significant increase in the levels of total protein and globulin without affecting albumin in sheep treated with zinc at concentrations of 30 and 60 mg/kg of feed for six weeks compared to the control group. Finally, Yusuf et al. (2023) noted a significant increase in the levels of total protein and globulin in the serum of goats treated with zinc at levels of 300 and 600 mg/kg of feed for 84 days compared to the control group.

2. The Effect of Co-Treatment with Enzyme Co-Q10 and Zinc Methionine on the Serum Lipid Profile in Awassi Sheep.

The statistical analysis results revealed no significant differences ($P \geq 0.05$) between the five groups during the initial 45 days of treatment in terms of cholesterol, triglycerides, high-density lipoprotein (HDL) cholesterol, low-density lipoprotein (LDL) cholesterol, and very-low-density lipoprotein (VLDL) cholesterol levels in the serum of Awassi sheep (Table 2).

To assess the biological impact of treatment with the enzyme cofactor Q10 and zinc methionine after 90 days (the second period) on the serum lipid profile, the obtained results, as shown in Table 2, indicated a significant decrease ($P \leq 0.05$) in cholesterol levels in animals treated with the fifth treatment group compared to the other treatments.

Regarding triglyceride levels, the results demonstrated a significant decrease ($P \leq 0.05$) in the serum of animals from the fifth treatment group compared to the other treatments. Additionally, the second, third, and fourth treatment groups showed a significant decrease ($P \leq 0.05$) in this parameter of this trait compared to the control group.

To make a tracking determination of the biological impact of treatment with the co-enzyme Q10 and zinc methionine on high-density lipoprotein (HDL) cholesterol levels, significant differences were observed after 90 days of treatment (the second period). The results showed a significant increase ($P \leq 0.05$) in HDL cholesterol levels in the fourth and fifth treatment groups compared to the control group. However, no significant differences were observed when compared to the second and third treatment groups.

Moreover, the table of Means 2 indicates a significant decrease ($P \leq 0.05$) in low-density lipoprotein (LDL) cholesterol levels during the second period in the fifth treatment group which showed a significant decrease in this parameter compared to the other treatments. The fourth treatment group also displayed a significant decrease ($P \leq 0.05$) compared to the control group while no significant differences were observed compared to the second and third treatment groups.

When reviewing the results for very-low-density lipoprotein (VLDL) cholesterol levels during the second period (90 days), a significant decrease ($P \leq 0.05$) was noted in the fifth treatment group, followed by the second, third, and fourth treatments compared to the control group. The same tables(2) demonstrate no significant differences ($P \leq 0.05$) in the atherogenic index during the first period (45 days of treatment). However, a significant decrease ($P \leq 0.05$) in the atherogenic index was observed in the fifth treatment group, followed by the third, fourth, and second treatments, compared to the control group after 90 days of treatment (the second period).

The results obtained from this study indicate a significant decrease in total cholesterol levels in the serum of treated Awassi lambs compared to the control group. This decrease could be attributed to the role of the co-enzyme Q10 in inhibiting the activity of Hydroxyl Methyl Glutaryl COA-reductase (HMGCR), which is responsible for cholesterol synthesis in the liver, by suppressing its mRNA receptors. Additionally, it may inhibit the activity of SREBP 2 in the liver, a key factor regulating cholesterol balance in the body, by stimulating genes responsible for cholesterol synthesis and controlling its entry into cells when its levels are low (Honda et al., 2010; Kamisoyama et al., 2010).

Zinc may also play a role in decreasing cholesterol levels by regulating fat metabolism in the liver through its influence on gene expression. This modulation of gene expression encodes enzymes involved in liver fat balance (Dieck et al., 2005). Zinc may also contribute to reducing cholesterol levels by affecting the expression of growth factors involved in fibrosis, inflammatory proteins, and apoptotic factors, all of which can increase cholesterol levels due to the damage to cell membranes in which the cholesterol is a formative participant (Piao et al., 2019). Another mechanism could involve zinc and zinc transporters increasing insulin storage and secretion, enhancing the phosphorylation of insulin receptor substrates to enhance connecting a series of cell signals and improving insulin sensitivity, which ultimately leads to lowering blood lipids through different pathways (Lynch et al., 2001).

Table 2: The Effect of Co-Treatment with Enzyme Co-Q10 and Zinc Methionine on the Serum Lipid Profile in Awassi Sheep.

Traits	Period	Treatments					significance
		First (T1)	Second (T2)	Third (T3)	Fourth (T4)	Fifth (T5)	
Cholesterol mg/dL	First (after 45 days)	109.12 ± 9.36 a	99.30 ± 8.62 a	108.99 ± 1.51 a	100.36 ± 2.72 a	106.13 ± 10.41 a	N. S
	Second (after 90 days).	119.24 ± 4.58 a	115.79 ± 2.00 a	112.99 ± 4.11 a	113.60 ± 2.85 a	85.25 ± 0.46 b	*
Triglycerides mg/dL	First (after 45 days).	71.01 ± 7.70 a	67.74 ± 1.33 a	57.02 ± 3.99 a	62.02 ± 5.42 a	55.60 ± 3.83 a	N. S

	Second (after 90 days).	68.15 ± 0.96 a	57.41 ± 1.64 b	54.07 ± 2.49 b	57.78 ± 0.96 b	41.11 ± 1.26 c	*
HDL Mg/dL	First (after 45 days).	50.82 ± 2.97 a	52.58 ± 1.94 a	54.18 ± 1.99 a	57.86 ± 3.22 a	55.03 ± 2.66 a	N. S
	Second (after 90 days)	52.57 ± 2.88 b	56.04 ± 0.63 ab	54.93 ± 0.93 ab	57.78 ± 0.90 a	58.83 ± 0.63 a	*
LDL Mg/dL	First (after 45 days).	44.10 ± 10.31 a	33.17 ± 9.25 a	43.41 ± 3.08 a	30.10 ± 4.52 a	39.98 ± 9.89 a	N. S
	Second (after 90 days)	53.04 ± 2.72 a	48.27 ± 1.83 ab	47.24 ± 3.73 ab	44.27 ± 2.61 b	18.20 ± 0.52 c	*
VLDL Mg/dL	First (after 45 days)	14.20 ± 1.54 a	13.55 ± 0.27 a	11.40 ± 0.80 a	12.40 ± 1.08 a	11.12 ± 0.77 a	N. S
	Second (after 90 days)	13.63 ± 0.19 a	11.48 ± 0.33 b	10.82 ± 0.50 b	11.56 ± 0.19 b	8.22 ± 0.25 c	*
Atherogenic Index	First (days after 45)	1.42 ± 0.20 a	1.29 ± 0.06 a	1.05 ± 0.05 a	1.08 ± 0.12 a	1.03 ± 0.11 a	N. S
	Second (after 90 days)	1.31 ± 0.06 a	1.03 ± 0.03 b	0.99 ± 0.04 b	1.00 ± 0.03 b	0.70 ± 0.02 c	*

The values represent means ± standard error. N.S. indicates no significant differences ($P \geq 0.05$). (*) indicates significant differences ($P \leq 0.05$). T1 = Control, T2 = Co-enzyme Q10 25 mg/animal, T3 = Co-enzyme Q10 50 mg/animal, T4 = Co-enzyme Q10 25 mg/animal + 150 mg/animal Zinc Methionine, T5 = Co-enzyme Q10 50 mg/animal + 150 mg/animal Zinc Methionine.

The decrease in triglyceride levels in the serum may be attributed to a synergistic role of zinc with the co-enzyme Q10 via zinc's entry into the structure and activation of Superoxide Dismutase (SOD), an enzyme that counteracts lipid peroxidation, which may prevent damage from free radicals and thus reducing triglyceride levels in the serum (Kucuk, 2008). Alternatively, zinc may activate nuclear receptor signals in adipose tissue cells, such as Peroxisome proliferator-activated receptor gamma (PPAR-γ), which plays a crucial role in lipid transport, metabolism, and activation of many genes involved in fat absorption, formation, and storage in adipocytes (Reiterer et al., 2004).

These results are consistent with the findings of Sadegzadeh-Sadat et al. (2021), who demonstrated a significant decrease in triglyceride and cholesterol levels in pregnant ewes treated with zinc at late pregnancy periods (30 and 300 mg/kg of feed) compared to the control group. They also align with the study by Hassan et al. (2021), which reported a significant decrease in cholesterol levels and no significant difference in triglyceride levels in young male rabbits treated with zinc (50 mg/kg feed) for 77 days. Witwit et al. (2021) observed a significant decrease in triglyceride and cholesterol levels and a significant increase in high-density lipoprotein in male and female patients with type 2 diabetes after treatment with zinc at a dose of 50 mg/day for 6 weeks, with no significant differences in low-density lipoprotein and very-low-density lipoprotein levels compared to the control group.

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