

RESEARCH PAPER

The Biological Effect of Treatment with Copper Oxide Nanoparticles and Garlic Oil on Some Biochemical Properties in the Blood of Awassi Lambs

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ABSTRACT

This study was conducted at the animal farm of the Department of Animal Production / College of Agriculture / Tikrit University, from 7/9/2025 to 7/12/2025, with the aim of studying the effect of adding copper oxide Nanoparticles and garlic oil on some biochemical characteristics in Awassi lambs. Twenty Awassi lambs, aged between 3-4 months and with an average weight of 26.10 ± 0.16 kg, were used and housed in a semi-open pen containing fixed feeders and waterers. The animals were randomly distributed into four treatments. The first treatment was treated with distilled water, while the second treatment was treated with copper oxide Nanoparticles at a concentration of 2 ml/animal/day (at a concentration of 20 mg), the third treatment was treated with garlic oil at a concentration of 1 ml/animal/day, and the fourth treatment was treated with a mixture of copper oxide Nanoparticles at a concentration of 2 ml/animal/day and garlic oil at a concentration of 1 ml/animal/day. The results of this study indicated that treatment with copper oxide Nanoparticles and garlic oil led to a significant increase ($P \geq 0.05$) in the total protein levels in the blood of animals in the fourth and third treatment groups compared to the first treatment group. Albumin and globulin levels showed no significant difference between the treated animals and the control group after 45 and 90 days of treatment. Meanwhile, serum glucose levels showed a significant decrease ($P \geq 0.05$) in the serum of animals in the fourth treatment group compared to the first and second treatment groups after 90 days of treatment. The results also showed a significant decrease ($P \geq 0.05$) in the total cholesterol level in the blood serum of animals treated in the fourth, third, and second treatments compared to the control group. The level of low-density lipoproteins also showed a significant decrease ($P \geq 0.05$) in the treated animals compared to the control group, while the level of high-density lipoproteins showed a significant increase ($P \geq 0.05$) in the fourth, third, and second treatments compared to the control group. However, no significant change occurred in the level of triglycerides and very low-density lipoproteins in the blood serum of animals treated in the treatment group and the control group after 90 days of treatment.

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INTRODUCTION

Sheep represent a strategic pillar of the livestock sector in Iraq, serving as a vital source of animal protein that is experiencing increasing demand due to current nutritional awareness [1]. The Awassi breed is considered the ideal choice due to its high productivity and meat quality, as well as its superior adaptation to harsh environmental and climatic conditions [2]. Based on the close relationship between performance efficiency and nutritional status, recent studies are focusing on enriching feed rations with non-traditional additives to enhance nutritional value and raise the animal's productivity and immunity levels [3].

Trace elements are of paramount importance in supporting animal health and productivity. Most sheep flocks require mineral supplements due to their low concentrations in basic rations or due to chemical interactions with antagonistic elements that limit their absorption. Copper (Cu) is considered the most important mineral in feed additive programs [4] because of its multiple vital and physiological roles within the body [5], which include regulating iron metabolism and transport, collagen synthesis, and activating antioxidant defense systems. In addition to being a pivotal element for the growth and development of the nervous system and overall growth, including wool and hair growth rates, bone and connective tissue formation, as well as enhancing immune response and reproductive efficiency [6,7], copper also contributes to regulating glucose and cholesterol metabolism pathways in the body [8]. Despite its crucial importance, copper absorption efficiency in ruminants remains relatively low under normal feeding conditions. This is due to the interaction of this metal with various rumen medium compounds such as sulfur, molybdenum, and iron, resulting in the synthesis of non-absorbable chemical complexes [9].

A deficiency of copper in the diet leads to health disorders including reduced reproductive efficiency, decreased sperm count and motility, increased embryonic mortality, slowed growth, low body weight, muscle weakness, anemia, skeletal deformities, connective tissue dysplasia, and myelin sheath disorders, resulting in neurological defects, lipid metabolism abnormalities, and heart failure. Conversely, research emphasizes the need to provide cattle with copper at optimal concentrations to meet their growth and developmental requirements [10,11]. While

copper deficiency is associated with pathological consequences [12], excessive intake is toxic, particularly in sheep [13]. It is worth noting that synthesizing metallic elements in nanoparticle form alters their physical properties and enhances their effective surface area for absorption, while maintaining their chemical composition unchanged [14]. Studies have confirmed a significant improvement in the bioavailability of micronutrients in ruminants when provided in nanoparticle form compared to conventional sizes [15,16]. In animal nutrition, several nano-metals, such as copper, zinc, selenium, and iron, have been used as feed additives to improve growth rates, production, and animal health. Research has demonstrated the superior bioavailability of copper oxide nanoparticles and their ability to improve copper status in ruminants compared to conventional copper oxide [17,18].

Medicinal plants, particularly garlic (*Allium sativum*), play a vital role in enhancing physiological and nutritional performance thanks to their richness in active chemical compounds such as sulfur compounds, allicin, and alliin. These compounds act as antifungal and antiparasitic agents, and their associated transformations also confer antibacterial effects, making them effective against a wide range of bacterial isolates. Furthermore, garlic contains compounds that induce the cyclooxygenase enzyme, which contributes to regulating serum cholesterol levels [19]. Garlic can also be used as a food to combat toxins resulting from heavy metals, which cause changes in body lipids due to oxidative stress [20]. Garlic oil has also demonstrated specific physiological effects by promoting growth and improving blood parameters [21,22], as well as reducing triglycerides in sheep serum [23]. It also contributes to supporting the antioxidant system and stimulating immunity [24,25]. Accordingly, small ruminants in the Iraqi environment suffer from a significant mineral deficiency (particularly copper and zinc) due to the soil's lack of these elements. This deficiency is caused by high sulfur concentrations, which exert an anti-agonistic effect, hindering the homeostasis of copper and zinc. This is reflected in the deficiency of these elements in forage plants grown in that soil and subsequently fed to the animals. Therefore, this study sought to investigate the effect of nano-copper oxide and garlic oil, individually or in combination, on some biochemical parameters of

the blood of local Awassi lambs.

MATERIALS AND METHODS

The experiment was conducted at the animal farm of the College of Agriculture/Department of Animal Production/Tikrit University from September 7, 2025, to December 7, 2025. Twenty locally raised Awassi lambs, aged 3-4 months and weighing an average of 0.1026 ± 0.16 kg, were used. The animals were divided into four treatments. The first treatment was given distilled water, the second treatment was given 2 ml/animal/day (at a concentration of 20 mg/ml) of copper oxide Nanoparticles, the third treatment was given 1 ml/animal/day of garlic oil, and the fourth treatment was given a mixture of 2 ml/animal/day of copper oxide Nanoparticles and 1 ml/animal/day of garlic oil. The animals underwent a 7-day preparatory period during which they received veterinary care to protect them from potential diseases. The animals were housed and fed individually in semi-open pens for each animal and were provided with their needs of concentrated feed with the components shown in Table 1 and supplied by Erbil Feed Company for Feeds, in two meals, morning and evening, in a quantity calculated on the basis of 3% of the animal's weight. This quantity is adjusted weekly according to the weight gain of each group, with the provision of dry roughage (hay) and the provision of drinking water continuously.

Sterile 5 ml syringes were used, containing 2 ml of copper oxide Nano-suspension (20 mg/ml concentration) and xanthan gum emulsion. Garlic oil was also prepared in 1 ml doses, along with the xanthan gum emulsion suspension. The doses were administered once per animal per day in the morning before feeding.

Preparation of the suspension

The suspension was prepared by gradually adding 5 grams of xanthan gum to each liter of

water and mixing at 10,000 rpm (low speed) for 3 minutes, followed by 30,000 rpm (high speed) for 5 minutes, until a colloidal solution was obtained.

Blood Tests

Blood samples were collected regularly every forty-five days at 7:30 AM after the animals had been deprived of feed for 12 hours, from the jugular vein in the neck region using a 10 ml wine syringe into clean, sterile wine plastic tubes and left to settle for one hour at laboratory temperature. After that, the tubes were stored in the refrigerator (4°C) at an angle (45°C) for 24 hours, and placed in a centrifuge for twenty minutes at a speed of 3000 rpm to separate the blood serum from the other components. The serum was stored in the freezer (-20°C) in sealed tubes until the biochemical tests were performed.

Estimation of Total Protein Concentration

Total serum protein concentration was estimated using a ready-made kit from the French company Biolabo by examining the samples with a spectrophotometer at a wavelength of 546 nm [26]. The following equation was used to calculate the total protein content:

$$\text{Total protein level (g/100 ml blood)} = \text{Sample absorbance} / \text{Standard solution absorbance} \times 6$$

Estimation of Albumin Concentration

Albumin concentration in serum was estimated using a ready-made kit from the French company Biolabo [27]. The following equation was applied to measure albumin levels:

$$\text{Albumin (g/100 ml)} = \text{Sample Absorbance} / \text{Standard Solution Absorbance} \times 5$$

Estimation of Globulin Concentration

To calculate the amount of globulin in serum, the following equation was applied, as described by Bishop et al. [28].

Table 1. Chemical Composition of the Experimental Feed.

Composition	Percentage %
Dry Matter	5.14
Total Protein	17.67
Ether Extract	2.1
Crude Fiber	6.74
Moisture	11.75
Energy (kcal)	2771

Total Cholesterol Concentration

The cholesterol concentration in serum was estimated using a ready-made kit from the French company BIOLABO by examining the samples with a spectrophotometer at a wavelength of 500 nm. To measure the cholesterol level in serum, the following equation was applied:

$$\text{Cholesterol Concentration (mg/dL)} = \text{Sample Absorbance} / \text{Standard Solution Absorbance} \times 200 \text{ (Standard Concentration)}$$

Triglyceride Concentration

The triglyceride level in serum was estimated using a ready-made kit manufactured by the French company BIOLABO by examining the samples with a spectrophotometer at a wavelength of 500 nm. To measure the triglyceride concentration in blood serum, the following equation was applied:

$$\text{Triglyceride (mg/dL)} = \text{Absorbance of sample} / \text{Absorbance of standard solution} \times 200 \text{ (standard concentration)}$$

High-density lipoproteins

The concentration of high-density lipoproteins in blood serum was measured using a ready-made kit manufactured by the French company BIOLABO by examining the samples with a spectrophotometer at a wavelength of 500 nm. To measure the concentration of high-density lipoproteins, the following equation was applied:

$$\text{HDL (mg/dL)} = \text{Absorbance of sample} / \text{Absorbance of standard solution} \times 100 \text{ (standard concentration)}$$

Low-density lipoproteins

The concentration of low-density lipoproteins in blood serum was estimated according to Friedewald et al. using the following equation [29]:

$$\text{LDL (mg/dL)} = \text{Total cholesterol} - (\text{High-density lipoproteins} + \text{Triglycerides} / 5)$$

Very Low-Density Lipoproteins (VLDL)

The concentration of very low-density lipoproteins (VLDL) in serum was calculated according to Friedewald et al. using the following formula [29]:

$$\text{VLDL (mg/dL)} = \text{Triglycerides} / 5$$

Estimation of AST Enzyme Concentration

To measure the activity of the AST enzyme in serum, a ready-made assay kit produced by Randox UK was used. The color intensity was measured at a wavelength of 505 nm IU/L [26].

Estimation of ALT Enzyme Concentration

This method relies on measuring the concentration of pyruvate hydrazone, which is formed from the reaction of pyruvate (a by-product of amino group transamination) with 2,4-dinitrophenyl hydrazene in the presence of ALT [30]. The absorbance was read at a wavelength of 546 nm.

Urea Concentration Estimation

The urea concentration in blood serum was estimated using a ready-made analytical kit (Kit) provided by Linear Chemicals, Spain. Samples were examined using a spectrophotometer at a wavelength of 600 nm [31]. To measure the urea level in blood serum, the following equation was applied:

$$\text{Urea (mg/100 ml blood)} = \text{Sample absorbance} / \text{Standard solution absorbance} \times 50$$

Creatinine Concentration Estimation

The creatinine concentration in blood serum was estimated using a ready-made analytical kit (Kit) provided by Linear Chemicals, Spain. The color intensity was measured at a wavelength of 490 nm [27], and the equation was applied to measure the creatinine concentration:

$$\text{Creatinine mg/L} = \text{Sample absorbance (second absorbance - first absorbance)} / \text{Standard solution absorbance (second absorbance - first absorbance)} \times 2$$

Statistical Analysis

Statistical analysis was performed using a one-way Complete Randomized Design (CRD). The significance of differences between treatments was tested using Duncan's multiple range test [32]. The SAS statistical analysis software [33] was used to analyze the data according to the following mathematical model:

$$Y_{ij} = \mu + T_i + e_{ij}$$

where: Y_{ij} = observation value j for treatment i ,

μ = overall mean for the studied trait, T_i = effect of treatment i , e_{ij} = experimental error with a normal and independent distribution, mean of zero, and equal variance of σ^{-2} .

RESULTS AND DISCUSSION

Effect of Dosing with Nano Copper Oxide and Garlic Oil on Serum Proteins and Glucose in Awassi Lambs

The results of the statistical analysis (Table 2) indicate a significant effect of treatment with Nano copper oxide and garlic oil on the total protein levels in the serum of Awassi lambs. The fourth treatment group showed a significant increase ($P \leq 0.05$) in this parameter compared to the control group, while the levels did not differ significantly from the other treatments. Table 2 also shows that serum albumin and globulin levels were not significantly affected by the different treatments. However, serum glucose levels showed a significant decrease ($P \leq 0.05$) in favor of the animals in the fourth treatment group compared to the other treatments after 45 days of treatment. To track the biological effect of treatment with Nano-copper oxide and garlic oil on total serum protein levels after 90 days of treatment, a significant increase ($P \leq 0.05$) was observed in animals treated with treatments 3 and 4 compared to the control group, while the difference was not significant compared to treatment 2. It was also noted that serum albumin and globulin levels were not significantly affected by the different treatments. As for serum glucose levels, the results showed a significant decrease ($P \leq 0.05$) in the blood of animals treated with treatment 4 compared to treatments 1 and 2,

while the difference was not significant compared to treatment 3.

The significant improvement observed in blood protein levels is attributed to the bioactive and stimulating properties of garlic. Garlic contains active sulfur compounds and potent antioxidants that contribute to improved protein digestion in the rumen and increased amino acid absorption in the intestines. Garlic also acts as an immune stimulant and enhances liver function, positively impacting the synthesis of blood proteins in the liver and their release into the bloodstream. Furthermore, garlic improves microbial protein synthesis in the rumen and increases the flow of absorbed amino acids in the small intestine [34,35].

This result is consistent with the findings of Kholif et al. [36] in dairy goats, El-Katcha et al. [37] in lambs, Hasan et al. [38] and Duvvu et al. [39] who reported significant increases in total protein levels in growing buffalo calves fed garlic, Sorang et al. [40] in Sahul calves, and El-Shereef [35] in rams.

The decrease in blood sugar levels observed in animals treated with treatment four (significantly) and treatment three (numerically) is attributed to the presence of allixin, the active compound in garlic. Allixin possesses a high capacity for carbohydrate oxidation and stimulates the body's cells to utilize glucose for energy, thus lowering its concentration in the bloodstream [41]. This may also be due to the role of sulfur and mosaic compounds in garlic, which contribute to stimulating insulin secretion or increasing cellular sensitivity to insulin. This leads to

Table 2. Effect of dosing with Nano-copper oxide and garlic oil on serum proteins and glucose in Awassi lambs.

Treatments	Blood serum proteins and sugars			
	Total protein (g/dL)	Albumin (g/dL)	Globulin (g/dL)	Blood glucose (mg/dL)
		After 45 days		
T1	6.38 ± 0.14 b	3.82 ± 0.26 a	2.56 ± 0.36 a	90.00 ± 7.97 a
T2	6.58 ± 0.10 ab	3.24 ± 0.08 a	3.34 ± 0.17 a	90.60 ± 1.63 a
T3	6.62 ± 0.19 ab	3.90 ± 0.28 a	2.72 ± 0.25 a	87.20 ± 1.46 a
T4	6.84 ± 0.02 a	4.00 ± 0.29 a	2.84 ± 0.29 a	71.20 ± 2.76 b
significant	*	N.S	N.S	*
		After 90 days		
T1	6.70 ± 0.13 b	3.34 ± 0.15 a	3.36 ± 0.22 a	81.20 ± 2.58 a
T2	6.98 ± 0.07 ab	3.82 ± 0.10 a	3.16 ± 0.07 a	86.20 ± 1.77 a
T3	7.06 ± 0.11 a	3.74 ± 0.20 a	3.32 ± 0.16 a	78.80 ± 3.80 ab
T4	7.12 ± 0.06 a	3.52 ± 0.21 a	3.60 ± 0.22 a	72.80 ± 0.80 b
significant	*	N.S	N.S	*

T1 = Control, T2 = 2 ml/animal/day of Nano-copper oxide, T3 = 1 ml/animal/day of garlic oil, T4 = 1 ml/animal/day of garlic oil + 2 ml/animal/day of Nano-copper oxide, N.S. = No significant difference ($P \geq 0.05$), * = Significant difference ($P \leq 0.05$), Number of lambs (5) per treatment, Values represent mean ± standard error

increased glucose uptake from the blood and its utilization by cells, thereby safely reducing serum glucose concentration within the animal's normal physiological limits [42]. The results of this study are consistent with those of Dosary [43], Usur [42], and Yuan et al. [44].

Effect of Dosing with Copper Oxide Nanoparticles and Garlic Oil on the Lipid Profile in Awassi Lambs

Statistical analysis results indicated a significant effect ($P \leq 0.05$) of treatment with copper oxide Nanoparticles and garlic oil on the levels of total cholesterol, high-density lipoproteins (HDL), and low-density lipoproteins (LDL) in the blood serum of Awassi lambs after 45 days of treatment (Table 3). Animals in treatments 3 and 4 showed a significant decrease ($P \leq 0.05$) in total cholesterol levels compared to the control group, while the level did not differ significantly from that in treatment 2. To track the biological effect of treatment with copper oxide Nanoparticles and garlic oil on HDL, its level showed a significant increase ($P \leq 0.05$) in the blood serum of animals in treatment 4 compared to the other treatments. From the same table, it is observed that the level of low-density lipoproteins showed a significant decrease ($P \leq 0.05$) in the blood serum of animals

in treatments 1 and 2. The third and fourth treatments were compared to the first and second treatments. As for the levels of triglycerides and very low-density lipoproteins, the treatment had no significant effect on their levels.

To monitor the biological effect of treatment with Nano-copper oxide and garlic oil on the lipid profile after 90 days of treatment, it was observed that the treated animals showed a significant decrease ($P \leq 0.05$) in total cholesterol levels compared to the control group. The fourth treatment also showed a significant increase ($P \leq 0.05$) in high-density lipoprotein (HDL) levels compared to the other treatments. From the mean Tables 4 and 5, it was noted that the concentration of low-density lipoprotein (LDL) cholesterol showed a significant decrease ($P \geq 0.05$) in the serum of the treated animals compared to the control group. Upon reviewing the results for triglycerides and very low-density lipoprotein (VLDL), it was observed that their levels did not differ significantly between the different treatments.

The decrease in total cholesterol and LDL levels may be attributed to the effect of copper, which is closely linked to lipid metabolism in the body. It contributes to regulating and reducing blood cholesterol levels through mechanisms

Table 3. Effect of dosing with Nano-copper oxide and garlic oil on the lipid profile in Awassi lambs.

Treatments	lipid profile				
	Total cholesterol (mg/dL)	Triglycerides (mg/dL)	HDL (mg/dL)	LDL (mg/dL)	VLDL (mg/dL)
After 45 days					
T1	119.80 ± 14.89 a	48.00 ± 1.52 a	42.00 ± 5.03 b	68.20 ± 15.57 a	9.60 ± 0.24 a
T2	97.40 ± 6.97 ab	53.20 ± 4.28 a	33.80 ± 0.58 b	51.20 ± 6.98 a	10.60 ± 0.87 a
T3	79.60 ± 5.15 b	50.00 ± 4.34 a	44.80 ± 8.97 b	23.00 ± 6.58 b	10.00 ± 0.84 a
T4	82.60 ± 4.17 b	46.00 ± 1.30 a	64.80 ± 2.15 a	10.80 ± 3.72 b	9.00 ± 0.32 a
Significant	*	N.S	*	*	N.S
After 90 days					
T1	121.00 ± 8.81 a	54.60 ± 2.23 a	41.00 ± 4.32 b	69.00 ± 10.69 a	11.00 ± 0.45 a
T2	83.80 ± 3.89 b	58.00 ± 4.36 a	37.80 ± 2.65 b	34.60 ± 3.85 b	11.60 ± 0.87 a
T3	83.60 ± 3.41 b	54.80 ± 4.03 a	43.60 ± 7.19 b	30.80 ± 4.18 b	11.00 ± 0.84 a
T4	97.00 ± 3.56 b	55.60 ± 2.56 a	59.00 ± 3.16 a	26.80 ± 5.91 b	11.20 ± 0.58 a
Significant	*	N.S	*	*	N.S

T1 = Control, T2 = 2 ml/animal/day of Nano-copper oxide, T3 = 1 ml/animal/day of garlic oil, T4 = 1 ml/animal/day of garlic oil + 2 ml/animal/day of Nano-copper oxide, N.S. = No significant difference ($P \geq 0.05$), *=Significant difference ($P \leq 0.05$), Number of lambs (5) per treatment, Values represent mean ± standard error

that reduce the synthesis of phospholipids and lipoproteins. This result is consistent with the findings of Samanta et al. [45], Zhang et al. [46], and Manimaran et al. [47]. This reduction may also be attributed to garlic, which has a lipid-lowering effect (hypolipidemic effect) resulting from the inhibition of key enzymes involved in fatty acid and cholesterol synthesis in the liver, such as HMG-CoA reductase [48,49]. In addition, garlic contributes to modifying the rumen environment to increase propionate production, which stimulates insulin secretion and glucose synthesis. Insulin possesses an anti-lipolytic effect, reducing the release of non-esterified fatty acids (NEFAs) from adipose tissue and thus decreasing their conversion in the liver to triglycerides and ketone bodies [36]. Furthermore, cholesterol levels are lowered by the pharmacological properties of garlic, which

inhibit the growth of harmful bacteria and reduce oxidative stress [37]. The results of this study are consistent with those of Dosary [43], Pirmohammadi et al. [50], El-Katcha et al. [37], Usur [42], El-Shereef [35], and Yuan et al. [44].

Effect of Dosing with Nano Copper Oxide and Garlic Oil on Some Kidney Function Indicators in Awassi Lambs

The results in Table 4 indicate that there were no significant differences ($P \geq 0.05$) between animals treated with Nano copper oxide and garlic oil and the control group in serum urea and creatinine levels in Awassi lambs after 45 days of treatment. However, the treatment had a significant effect on serum urea levels after 90 days of treatment. Animals in the fourth treatment group showed a significant decrease ($P \geq 0.05$) in this value

Table 4. Effect of dosing with Nano-copper oxide and garlic oil on some kidney function indicators in Awassi lambs.

Treatments	Kidney function indicators	
	(Blood urea (mg/dL)	Creatinine (mg/dL)
	After 45 days	
T1	28.40 ± 1.50 a	0.78 ± 0.09 a
T2	28.80 ± 1.11 a	0.76 ± 0.07 a
T3	25.00 ± 1.76 a	0.64 ± 0.06 a
T4	24.80 ± 2.03 a	0.68 ± 0.07 a
Significant	N.S	N.S
Treatments	Kidney function indicators	
	(Blood urea (mg/dL)	Creatinine (mg/dL)
	After 90 days	
T1	44.40 ± 2.14 a	0.78 ± 0.04 a
T2	44.20 ± 1.98 a	0.74 ± 0.05 a
T3	40.20 ± 1.85 ab	0.72 ± 0.06 a
T4	36.20 ± 0.73 b	0.68 ± 0.04 a
Significant	*	N.S

T1 = Control, T2 = 2 ml/animal/day of Nano-copper oxide, T3 = 1 ml/animal/day of garlic oil, T4 = 1 ml/animal/day of garlic oil + 2 ml/animal/day of Nano-copper oxide, N.S. = No significant difference ($P \geq 0.05$), *=Significant difference ($P \leq 0.05$), Number of lambs (5) per treatment, Values represent mean ± standard error

Table 5. Effect of dosing with Nano-copper oxide and garlic oil on some liver function indicators in Awassi lambs.

Treatments	Liver function indicators	
	AST (International Units/L)	ALT (International Units/L)
	After 45 days	
T1	64.80 ± 1.16 a	15.80 ± 0.66 a
T2	60.40 ± 3.03 ab	15.20 ± 0.37 a
T3	57.60 ± 1.40 b	15.00 ± 0.55 a
T4	58.40 ± 1.75 b	14.80 ± 0.37 a
Significant	*	N.S
Treatments	Liver function indicators	
	AST (International Units/L)	ALT (International Units/L)
	After 90 days	
T1	64.20 ± 1.53 a	19.80 ± 1.32 a
T2	63.20 ± 1.39 a	18.00 ± 1.14 a
T3	61.40 ± 1.12 ab	17.20 ± 0.66 ab
T4	57.40 ± 2.34 b	14.60 ± 0.24 b
Significant	*	*

T1 = Control, T2 = 2 ml/animal/day of Nano-copper oxide, T3 = 1 ml/animal/day of garlic oil, T4 = 1 ml/animal/day of garlic oil + 2 ml/animal/day of Nano-copper oxide, N.S. = No significant difference ($P \geq 0.05$), *=Significant difference ($P \leq 0.05$), Number of lambs (5) per treatment, Values represent mean ± standard error

compared to the first and second treatments, while it did not differ significantly from the third treatment group. Creatinine levels did not show any significant differences between the different treatments during this period.

The significant decrease in urea levels observed in animals treated with treatment four, and the non-significant decrease in blood urea levels in animals treated with treatment three, may be attributed to the effect of active compounds in garlic on rumen macrobiotic. These compounds reduce ammonia production within the rumen by decreasing the activity of amino acid-degrading bacteria, leading to increased nitrogen utilization efficiency in the rumen and a reduction in nitrogen loss as urea in the blood and urine [42]. This finding is consistent with the results of El-Shereef [35], Dey et al. [51], and Yuan et al. [44].

Effect of dosing with Nano-copper oxide and garlic oil on some liver function indicators in Awassi lambs

The results of the statistical analysis shown in Table 5 showed a significant effect of treatment with Nano-copper oxide and garlic oil on the concentration of the aminotransferase enzyme and AST between the different treatments, as a significant decrease ($P \leq 0.05$) was recorded in the blood serum of animals in the third and fourth treatments compared to the control group. As for the ALT enzyme, the treatment did not have any significant effect on its level after 45 days of treatment. To monitor the AST and ALT enzyme levels after 90 days of treatment, a significant decrease ($P \leq 0.05$) was observed in the serum AST level of animals in the fourth treatment compared to the other treatments.

Similarly, the ALT enzyme level also showed a significant decrease ($P \leq 0.05$) in the serum of animals in the fourth treatment compared to the first and second treatments, while it did not differ significantly from the third treatment. ALT and AST enzymes are very sensitive indicators of liver cell health; any damage or stress to liver cells leads to the leakage of these enzymes into the bloodstream and an increase in their concentration. The decrease in these enzymes in the treatments fed garlic oil indicates the hepatoprotective effect of allicin, a compound found in garlic, and the protection of liver cell membranes from damage thanks to its antioxidant organosulfur compounds [40]. These results are consistent with what

Vaswani et al. [8] and Usur [38] found.

CONCLUSION

The results showed a significant increase in total blood protein levels in animals treated in both the third and fourth treatments, without affecting albumin and globulin. The fourth treatment also resulted in a significant decrease in glucose, along with a decrease in total cholesterol and low-density lipoprotein (LDL) cholesterol, compared to an increase in high-density lipoprotein (HDL) cholesterol in the treated groups compared to the control group. Triglyceride and very low-density lipoprotein (VLDL) cholesterol levels were not significantly affected between the groups. These results indicate a positive effect of the supplementation on protein and lipid profiles without negative effects on the metabolic health of the lambs.

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CONFLICT OF INTEREST

The authors declare that there is no conflict of interests regarding the publication of this manuscript.

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