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Effect of treatment with copper oxide Nanoparticles and garlic oil on some productive and hematological traits in 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 September 7, 2025, to December 7, 2025. The aim was to investigate the effect of adding copper oxide Nanoparticles and garlic oil on some productive and hematological traits in Awassi lambs. Twenty Awassi lambs, aged 3-4 months with an average weight of 26.10 ± 0.16 kg, were used. They were housed in a semi-open pen with fixed feeders and waterers. The animals were randomly divided into four treatments. The first treatment was treated with distilled water, the second treatment with copper oxide Nanoparticles at a concentration of 2 ml/animal/day (at a concentration of 20 mg), the third treatment with garlic oil at a concentration of 1 ml/animal/day, and the fourth treatment 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 a significant improvement ($P \leq 0.05$) in the final weight, daily weight gain, and total weight gain of the lambs treated in the fourth treatment compared to the first and second treatments. The animals in the fourth treatment also recorded a significant increase in the number of red blood cells, blood hemoglobin concentration, and packed red blood cell volume compared to the control group after 90 days of treatment. No significant differences were found in the values of red blood cell indices (MCV, MCH, and MCHC) between the four groups. The results also showed a significant decrease ($P \leq 0.05$) in the total white blood cell count and neutrophil count in the blood of the animals in the fourth, third, and second treatments compared to the control group. The percentage of lymphocytes increased significantly ($P \leq 0.05$) in the blood of the treated animals compared to the control group after 45 and 90 days of treatment. As for the percentage of Eosinophil's, basophils, and monocytes, the treatment with copper oxide Nanoparticles and garlic oil had no significant effect on their percentage.

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1. INTRODUCTION

Sheep are a cornerstone of livestock production, and their products, such as red meat and milk, contribute significantly to the national agricultural income. They constitute 53% of the total livestock population in Iraq, numbering 677,217 head. The Awassi sheep breed is distinguished by its high productivity and remarkable tolerance to the harsh environmental and climatic conditions prevalent in the regions where it is raised [1]. Maintaining optimal production performance and good physiological condition in sheep is crucial for the sustainability of breeding operations [2, 3].

Trace elements are essential for animal health and productivity, so most sheep receive some form of mineral supplementation due to their low concentration in feed rations or interference from antinutrients that inhibit their absorption. Copper (Cu) is one of the most commonly added elements [4] because of its vital importance and its role in numerous physiological and biochemical functions within the body [2]. These functions include iron metabolism and transport, collagen synthesis, and antioxidant defense mechanisms. Copper is also essential for nervous system development, growth (including wool and hair growth), bone and connective tissue formation, and supporting immunity and fertility [5, 6]. It contributes to regulating glucose and cholesterol metabolism in the body [7]. Despite its importance, copper absorption in ruminants is relatively low under typical feeding conditions. This is due to copper reacting with various substances in the rumen, such as sulfur, molybdenum, and iron, resulting in the formation of non-absorbable compounds [8]. A deficiency of copper supplements in the diet can cause reproductive disorders, sperm development, increased fetal mortality, slow growth, and decreased body weight. Moreover, it may lead to muscle weakness, anemia, bone deformities, malformed connective tissue synthesis, myelin sheath dysfunction of nerve tissues, neurological defects, changes in lipid metabolism, and heart failure. However, it has been established that copper should be provided to cattle at optimal concentrations and according to the animal's growth and development requirements [9, 10]. Although copper deficiency causes health problems [11], excessive consumption can be toxic, especially in sheep [3].

The addition of metallic elements in the form of Nanoparticles alters their physical properties and increases their surface area for absorption, while the chemical properties of the metal remain unchanged [12]. Studies have indicated a significant improvement in the bioavailability of trace elements in ruminants when presented as Nanoparticles compared to larger sizes [13, 14]. In animal nutrition, various Nano-metals, such as copper, zinc, selenium, and iron, have been used as feed supplements to improve animal growth, production, and health. Studies have shown that Nano-copper oxide exhibits higher bioavailability and a greater ability to improve copper status in ruminants compared to conventional copper oxide [15, 16].

Medicinal plants, including garlic (Allium sativum), play a significant role in promoting physiological and nutritional status due to their content of various chemical compounds such as sulfides, allicin, and alliin. These compounds act as antifungal and antiparasitic agents. Furthermore, the transformation of these compounds gives garlic its antibiotic properties, making it effective against many different types of bacteria. Garlic also contains compounds that activate cyclooxygenase, an enzyme involved in regulating serum cholesterol levels [17]. Garlic can be used as a food to combat toxins resulting from heavy metals, which cause changes in body lipids due to the presence of oxidative agents [18]. The use of garlic oil also has important biological effects; it promotes growth and improves blood parameters [19, 20]. It reduces triglyceride levels in sheep blood serum [21]. It also contributes to antioxidant support and immune system stimulation [22, 23]. Based on the above, small ruminants in the Iraqi environment suffer from a deficiency of mineral elements (especially copper and zinc) due to the soil's lack of these elements, primarily because of the high sulfur levels in the soil, which conflict with the presence of copper and zinc due to the anti-agonistic effect. This leads to a deficiency of these elements in crops grown in that soil, which will later be used as fodder. Therefore, this study aimed to investigate the effect of Nano-copper oxide and garlic oil, individually or in combination, on the productive and physiological performance of local Awassi lambs.

2. 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 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 experimental 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, 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 continuous provision of drinking water.

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

Sterile medical syringes with a capacity of 5 ml were used, into which 2 ml of copper oxide Nano-suspension (at a concentration of 20 mg) and xanthan emulsion were placed. Garlic oil was also prepared with a quantity of 1 ml and the xanthan emulsion suspension. The doses were given at a rate of one dose/animal/day in the morning before the feed was provided.

2.1. Preparation of the suspension

The suspension was prepared by gradually adding 5 grams of xanthan per liter of water and mixing it at a speed of 10,000 rpm (low speed) for 3 minutes, and then at a speed of 30,000 rpm (high speed) for 5 minutes until a colloidal solution was obtained.

2.2. Blood tests

Blood samples were collected regularly every forty-five days at 7:30 a.m., after the animals had been fed for 12 hours, from the jugular vein in the neck region using a 5 ml wine syringe into clean, sterile wine plastic tubes.

2.2.1. Red blood cell count

Red blood cell counts were performed using a hemocytometer. Blood was drawn with a pipette to the 0.5 mark, and then the volume was increased to the 101 mark with Hymen's solution. After mixing the contents of the pipette by shaking it by hand for ten seconds, the counting slide was prepared by discarding the first three drops of blood and using the fourth drop by placing it on the edge of the cover slide. The cells were left for two minutes to settle and then examined under a light microscope (40x magnification) by counting the cells in five medium squares, each medium square containing 16 sub squares [24]. The total number of red blood cells is calculated using the following equation:

RBC (mm3 blood) = Number of cells in five medium squares × 200 (dilution correction factor) × 50 (volume correction factor).

2.2.2. Packed cell volume

The percentage of packed cell volume was measured by placing open-ended glass capillary tubes, free of anticoagulant, in blood at a 45° angle. After the tube was three-quarters full, the ends submerged in blood were sealed with synthetic clay. The tubes were then centrifuged in a micro-hematocrit centrifuge for five minutes at 12,000 rpm. The length of the hematocrit in the capillary tube was read using a hematocrit reader. This length represents the percentage of packed red blood cell volume [24].

2.2.3. Hemoglobin concentration

Hemoglobin concentration was measured spectrophotometrically using an Apel spectrophotometer at a wavelength of 540 nm, according to the Drabkin and Austin method [25], following the instructions of Randox Laboratories Co. Ltd., Antrum, United Kingdom [26]. The hemoglobin concentration was calculated using the following equation:

Hb (g/100 ml of blood) = Spectrophotometer reading / Standard solution reading × Dilution factor × Concentration of standard hemoglobin solution / 1000.

2.2.4. Red blood cell indices

It was calculated according to the following equations:

MCV (cubic micrometers) = (PCV / number of RBC) × 10

MCH (picograms) = (Hb / number of RBC) × 10

MCHC (%) = (Hb / PCV) × 100

2.2.5. White blood cell count

White blood cells were counted using a hemocytometer by drawing blood with a pipette to the 0.5 mark, then filling the volume to the 11 mark with Turk's solution. After mixing the contents by shaking the solution for ten seconds, the sample was left to stand for three minutes to stain the white blood cell nuclei. To prepare the counting slide, the first three drops were discarded, and the fourth was used by placing it on the edge of the slide's contact with its cover. It was left for two minutes to allow the cells to settle and stabilize, then examined under a light microscope (40x magnification) by counting the cells within the large square [27]. The total white blood cell count was calculated using the following equation: White blood cell count / mm³ blood = Number of cells in the square Central bulk × 20 (dilution correction factor) × 10 (volume correction factor).

2.2.6. Differential white blood cell count

The differential white blood cell count was calculated by taking a blood smear. A small drop of blood was placed on the edge of a glass slide, which was then spread by drawing another slide over it. The smear was left to dry for ten minutes and then fixed by dipping it in methyl alcohol (0.97N). The slides were then stained by dipping them for half an hour with the pre-prepared Gamsa stain (Appendix 2). The smears were then gently washed with tap water to remove excess stain. The number and types of white blood cells (lymphocytes, monocytes, neutrophils, eosinophils, and basophils) were examined under a light microscope using an oil immersion lens (100x) by counting 100 white blood cells while moving the slide along a Z-axis [28].

2.3. 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 [29]. The SAS statistical analysis software [30] was used to analyze the data according to the following mathematical model:

$Yij = \mu + Ti + eij$

where:

Yij = observation value j for treatment i.

μ = overall mean for the studied trait.

Ti = effect of treatment i.

eij = experimental error with a normal and independent distribution, mean of zero, and equal variance of σ^{-2}.

3. RESULTS AND DISCUSSION

3.1. Effect of Dosing with Nano Copper Oxide and Garlic Oil on Some Productive Performance Indicators in Awassi Lambs

The results in Table 2 indicate no significant differences ($P \leq 0.05$) between treatments for the initial weight of Awassi lambs. However, a significant effect ($P \leq 0.05$) was recorded for the treatment with Nano copper oxide and garlic oil on the final weight between the groups. Animals in the fourth treatment showed a significant increase ($P \leq 0.05$) compared to the control group (first), while the results did not differ significantly from the second and third treatments. As shown in Table 2, the treatment with Nano-copper oxide and garlic oil had a significant effect on the four groups of Awassi lambs in terms of total and daily

weight gain. Animals in the fourth treatment group showed a significant improvement ($P \leq 0.05$) compared to the first and second treatments, while the third treatment group showed no significant difference. No significant differences were found in the relative growth rate between the different treatments. The significant improvement in final weight, total weight gain, and daily weight gain in the fourth treatment group may be attributed to the synergistic effect of garlic oil and copper. Garlic improves the intestinal environment and gut macrobiotic. Gram-positive harmful bacteria exhibit a very high sensitivity to the antibacterial compounds found in garlic, compared to beneficial intestinal bacteria, which are unaffected by garlic due to their low sensitivity to it. In addition, garlic possesses an effect similar to natural prebiotics due to its fructo-oligosaccharide classification [31].

Table 2. Effect of dosing with Nano-copper oxide and garlic oil on some productive performance indicators in Awassi lambs

Treatments	Productivity performance indicators				
	Initial weight (kg)	Final weight (kg)	Total weight gain (kg)	Daily weight gain (g)	Growth rate (%)
T1	**26.00 ± 0.32 a**	**52.20 ± 0.58 b**	**26.20 ± 0.86 b**	**321.61 ± 9.58 b**	**101.40 ± 4.09 a**
T2	**26.20 ± 0.37 a**	**52.80 ± 1.71 ab**	**26.60 ± 1.44 b**	**316.66 ± 17.09 b**	**101.60 ± 4.83 a**
T3	**26.20 ± 0.37 a**	**54.00 ± 0.45 ab**	**27.80 ± 0.58 ab**	**330.95 ± 6.94 ab**	**106.40 ± 3.49 a**
T4	**26.00 ± 0.32 a**	**55.60 ± 0.81 a**	**29.60 ± 0.68 a**	**352.38 ± 8.07 a**	**113.80 ± 2.63 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 ($P \leq 0.05$)
=* Significant difference ($P \leq 0.05$)
Number of lambs (5) per treatment
Values represent mean ± standard error

Furthermore, the volatile oils in garlic modulate rumen fermentation, inhibiting deamination and directly reducing methane production, thus improving energy and protein utilization efficiency [32–34]. Moreover, the essential oils and active compounds in garlic possess appetite-stimulating and digestive properties [35]. Furthermore, the active compounds in garlic stimulate the synthesis of vitamin B6, which improves digestion. Garlic also plays a role in stimulating the synthesis of microbial proteins and volatile fatty acids, and reducing the number of parasites in the intestines, thus enhancing nutrient absorption [36–40] (Gu et al., 2024). On the other hand, copper may play an important role in improving the immune status of treated animals, as copper plays a vital role in maintaining the efficiency and development of the immune system by raising the level of antibodies (Ig) and activating phagocytes and lymphocytes, thus enhancing the animal's humoral immunity [41, 42]. These results are consistent with those of Usur [35], Dey et al. [43], Rabee et al. [40], and Yuan et al. [44].

3.2. Effect of Dosing with Nano Copper Oxide and Garlic Oil on Some Physical Indicators of Awassi Lamb Blood

The results of the statistical analysis showed no significant differences between the four treatments of Awassi lambs regarding the effect of Nano copper oxide and garlic oil treatment on red blood cell counts. However, the fourth treatment showed a significant decrease in white blood cell counts compared to the control group, while it did not differ significantly from the second and third treatments. The same table also shows a significant increase in hemoglobin levels in the animals of the third and fourth treatments compared to the control group, while it did not differ significantly from the second treatment. The results also indicated a significant increase in the percentage of hematocrit in the blood of the animals of the third treatment compared to the first treatment, while it did not differ significantly from the second and fourth

treatments after 45 days of treatment. During the second period (90 days), a significant effect ($P \leq 0.05$) of treatment with Nano-copper oxide and garlic oil was observed in red blood cell counts and the percentage of packed cell volume between treatments. A significant increase ($P \leq 0.05$) in their values was noted in the blood of animals in the fourth treatment compared to the other treatments. White blood cells also showed a significant decrease ($P \leq 0.05$) in favor of animals in the third and fourth treatments compared to the first treatment, while it did not differ significantly from the second treatment. In the same context, animals treated with Nano-copper oxide and garlic oil showed a significant increase ($P \leq 0.05$) in hemoglobin concentration compared to the control group, while it did not differ significantly from the second and third treatments during this period (Table 3).

The significant increase in red blood cell count, hemoglobin, and hematocrit in the fourth treatment animals and the non-significant increase in the animals that received Nano-copper and garlic oil may be due to copper, which is an essential element involved in the structure of the ceruloplasmin protein. The latter plays a vital and crucial role in the oxidation of ferrous iron to ferrous iron, which is necessary for the binding of iron to transferrin for its transport and use by the bone marrow in the manufacture of hemoglobin and red blood cells [45]. This may also be attributed to the ability of garlic oil to stimulate hemoglobin synthesis by inducing temporary local hypoxia: According to Iranloye's study [46], garlic extract acts as an active oxygen scavenger. The active components of garlic compete with hemoglobin for oxygen within red blood cells, causing a state of "temporary relative hypoxia" in tissues, which is the primary physiological trigger for the release of erythropoietin, which stimulates

Table 3. The effect of dosing with Nano-copper oxide and garlic oil on some physical indicators of the blood of Awassi lambs

Treatments	Physical indicators of blood			
	Red blood cells (x 10^6)	White blood cells (10^3)	Hemoglobin (g/dL)	Hematocrit (%)
		After 45 days		
T1	10.60 ± 0.24 a	6.02 ± 0.49 a	11.20 ± 0.20 b	34.20 ± 0.20 b
T2	10.60 ± 0.40 a	5.20 ± 0.20 ab	11.80 ± 0.20 ab	35.20 ± 0.58 ab
T3	11.20 ± 0.20 a	5.60 ± 0.24 ab	12.20 ± 0.20 a	36.00 ± 0.55 a
T4	11.60 ± 0.40 a	4.80 ± 0.37 b	12.40 ± 0.24 a	35.60 ± 0.60 ab
significant	N.S	*	*	*
		After 90 days		
T1	10.60 ± 0.24 b	6.68 ± 0.36 a	10.20 ± 0.37 b	31.60 ± 0.87 b
T2	10.80 ± 0.37 b	5.76 ± 0.32 ab	11.00 ± 0.32 ab	32.80 ± 0.97 b
T3	11.20 ± 0.37 b	5.48 ± 0.39 b	11.00 ± 0.32 ab	33.20 ± 1.16 b
T4	12.40 ± 0.24 a	4.86 ± 0.26 b	12.00 ± 0.32 a	0.37 ± 36.20 a
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 ($P \leq 0.05$)
=* Significant difference ($P \leq 0.05$)
Number of lambs (5) per treatment
Values represent mean ± standard error

the bone marrow to produce more red blood cells and accelerate hemoglobin synthesis. On the other hand, it improves the functions of hematopoietic organs and supports the immune system. According to a study by Li et al. [47], the bioactive compounds in garlic enhance the immune system and improve the efficiency of vital organs responsible for blood cell formation and

development, such as the thymus, spleen, and bone marrow. This ultimately leads to increased red blood cell production and improved hematological parameters in animals [48].

The significant decrease in white blood cell count may be attributed to garlic oil, which contains bioactive volatile sulfur compounds, primarily allicin and agoene. These compounds act as potent natural antibiotics and immune system stimulants, increasing the body's resistance to diseases and infections and stimulating cellular immunity [49, 50]. These results are consistent with those of Dosary [50] and Sorang et al. [48].

3.3. Effect of Dosing with Copper Oxide Nano and Garlic Oil on Red Blood Cell Indices in Awassi Lambs

The results of the statistical analysis shown in Table 4 indicate no significant differences in red blood cell volume (MCV), mean corpuscular hemoglobin (MCH), or mean corpuscular hemoglobin concentration (MCHC) at a significance level of ($P \geq 0.05$) between animals treated with copper oxide Nano and garlic oil and the control group after 45 and 90 days of treatment.

Table 4. Effect of dosing with Nano-copper oxide and garlic oil on red blood cell indices in Awassi lambs

Treatments	Red blood cell indicators		
	MCV (in femtoliters)	MCH (pg)	MCHC (g/dL)
	After 45 days		
T1	**32.40 ± 0.67 a**	**10.60 ± 0.24 a**	**32.40 ± 0.40 a**
T2	**33.40 ± 1.17 a**	**11.20 ± 0.37 a**	**33.20 ± 0.20 a**
T3	**32.40 ± 0.24 a**	**11.00 ± 0.01 a**	**33.60 ± 0.24 a**
T4	**31.00 ± 1.00 a**	**10.80 ± 0.20 a**	**34.60 ± 0.60 a**
Significant	N.S	N.S	N.S
	After 90 days		
T1	**29.80 ± 0.58 a**	**9.60 ± 0.40 a**	**32.00 ± 1.38 a**
T2	**30.40 ± 0.68 a**	**10.20 ± 0.20 a**	**33.20 ± 0.58 a**
T3	**29.80 ± 0.73 a**	**9.80 ± 0.20 a**	**32.80 ± 0.37 a**
T4	**29.20 ± 0.73 a**	**9.60 ± 0.40 a**	**32.80 ± 0.66 a**
Significant	N.S	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 ($P \leq 0.05$)
=* Significant difference ($P \leq 0.05$)
Number of lambs (5) per treatment
Values represent mean ± standard error

3.4. Effect of Dosing with Copper Oxide Nanoparticles and Garlic Oil on White Blood Cell Differential Count in Awassi Lambs

The results of the statistical analysis (Table 5) showed a significant effect ($P \leq 0.05$) of the treatment with copper oxide Nanoparticles and garlic oil on the percentage of neutrophils and lymphocytes in the blood of Awassi lambs. The treated animals showed a significant decrease ($P \leq 0.05$) in the percentage of neutrophils compared to the control group, while the treated animals showed a significant increase ($P \leq 0.05$) in the percentage of lymphocytes compared to the control group. As for basophils, eosinophil's, and monocytes, no significant differences were observed in their percentages between the different treatments after 45 days of treatment. To monitor the biological effect of the treatment with copper oxide Nanoparticles and garlic oil on the white blood cell differential count after 90 days of treatment, the animals in the second, third, and fourth treatments showed a decrease. Significantly ($P \leq 0.05$) the percentage of neutrophils in their

blood was higher compared to the control group. From the same table, it is noted that the percentage of lymphocytes witnessed a significant increase ($P \leq 0.05$) in the blood of the treated animals compared to the first treatment. As for basophils and eosinophil's, no significant differences were recorded in their percentage between the different treatments after 90 days of treatment.

Table 5. Effect of dosing with Nano-copper oxide and garlic oil on the differential white blood cell count in Awassi lambs

Treatments	White blood cell differential count(%)				
	Neutrophils	Basophils	Eosinophil's	Lymphocytes	Monocytes
			After 45 days		
T1	37.20 ± 0.37 a	0.60 ± 0.24 a	5.40 ± 0.24 a	54.00 ± 0.32 b	2.80 ± 0.20 a
T2	35.00 ± 0.45 b	0.60 ± 0.24 a	5.40 ± 0.24 a	56.40 ± 0.60 a	2.60 ± 0.24 a
T3	34.40 ± 0.51 b	0.60 ± 0.24 a	5.20 ± 0.20 a	57.00 ± 0.45 a	2.80 ± 0.20 a
T4	34.00 ± 0.32 b	1.00 ± 0.01 a	5.20 ± 0.20 a	57.00 ± 0.32 a	2.80 ± 0.20 a
Significant	*	N.S	N.S	*	N.S
			After 90 days		
T1	37.00 ± 0.71 a	0.80 ± 0.10 a	6.94 ± 0.27 a	50.12 ± 0.81 b	4.96 ± 0.20 a
T2	32.40 ± 0.60 b	0.52 ± 0.04 a	7.02 ± 0.12 a	55.04 ± 0.46 a	5.02 ± 0.11 a
T3	33.80 ± 0.97 b	0.60 ± 0.10 a	6.48 ± 0.35 a	53.90 ± 0.74 a	5.08 ± 0.23 a
T4	34.40 ± 0.68 b	0.80 ± 0.08 a	6.72 ± 0.14 a	53.22 ± 0.59 a	4.74 ± 0.25 a
Significant	*	N.S	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 ($P \leq 0.05$)
=* Significant difference ($P \leq 0.05$)
Number of lambs (5) per treatment
Values represent mean ± standard error

The increase in lymphocytes and decrease in neutrophils demonstrates copper's ability to stimulate white blood cells, antibodies, and the production of antioxidant enzymes [51]. Copper Nanoparticles play a positive role in enhancing the immune response due to their high bioavailability and ease of cellular penetration compared to conventional copper [15, 16]. Furthermore, garlic contains bioactive volatile sulfur compounds, such as allicin and ajoene, which act as potent natural antibiotics and immune system stimulants, thus increasing the body's resistance to diseases and infections and stimulating cellular immunity [49, 50]. Furthermore, the stability of basophils, which play a crucial role in immune surveillance and histamine release during allergic reactions, with their values remaining normal and low, demonstrates the strength of the lambs' immune system and the stability of their antioxidant activity. This stability is attributed to the support provided by copper and garlic oil to antioxidant enzymes, thus maintaining the animals' overall health. Additionally, the stability of eosinophil's, which are essential active cells in the defense against parasitic infections and are activated in allergic conditions, with their values remaining normal, confirms that the lambs in the experiment were completely free of parasitic infections, allergic reactions, and allergic inflammation.

4. CONCLUSIONS

This study showed that the addition of Nano-copper oxide and garlic oil significantly improved the final weight and weight gain of lambs. The red blood cell count also improved, with increases in red blood cell count, hemoglobin, and packed cell volume, without affecting the red blood cell indices (MCV, MCH, MCHC). White blood cell counts showed a significant decrease in total and neutrophil counts, while lymphocytes showed a significant increase, reflecting an improved immune response. The proportions of eosinophil's, basophils, and monocytes were unaffected, indicating the safety of these additives.

DECLARATIONS

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Authors' Contributions

Ban Talal Ahmed: Data collection, research, original drafting, formal analysis, methodology, and project management.

Abdul Khaliq Ahmed Farhan Al-Janabi: Conceptualization, research, supervision, verification, review writing, and editing.

Conflict of Interest

The authors declare that there is no conflict of interest, whether financial, personal, or otherwise, with any other individuals or organizations related to the material discussed in this study.

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