Effect of adding nano zinc and protected amino acids (methionine and lysine) on some physiological characteristics of Awassi lambs

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Abstract

This study aimed to investigate Effect of treatment with nano zinc (ZNO) and protected amino acids (methionine and lysine) on some physiological parameters in Awassi lambs. Thirty-two lambs, aged between 4 and 5 months and with an average weight of 26.56 ± 0.20 kg, were randomly divided into eight groups (4 animals per group). The study included two 60-day periods. Animals in the first group (control) were treated with 10 ml of distilled water (per animal), while animals in the second treatment were treated with 2 g/head per day of methionine and 4 g/head per day of lysine. Animals in the fourth treatment were treated with a mixed dose of methionine and lysine (2 and 4 g/head per day, respectively), while animals in the fifth treatment were treated with 15 mg/head per day of nano zinc. While the sixth treatment was dosed with methionine (2 g/head per day) + nano zinc (15 mg/head per day). The seventh treatment was dosed with lysine (4 g/head per day) + nano zinc (15 mg/head per day). The eighth treatment was dosed with methionine and lysine (2 and 4 g/head per day) respectively + nano zinc (15 mg/head per day). The results of this study indicated a significant improvement ($P \geq 0.05$) in the number of red blood cells, hemoglobin concentration and packed cell volume in the blood of animals in the eighth treatment (nano zinc, methionine and lysine mixture) compared to the control group after 60 days of treatment. The second period (120 days) also witnessed a significant increase ($P \geq 0.05$) in the number of red blood cells and hemoglobin level in the blood of animals in the eighth treatment compared to the first, second and third treatments. In the same context, the treatment of nano zinc, methionine and lysine mixture showed a significantly excelled in the percentage of packed cell volume in favor of the eighth, seventh and sixth treatments compared to the rest of the treatments. The results also showed a significant decrease ($P \geq 0.05$) in the total white blood cell count in the blood of animals in the sixth, seventh, and eighth treatments compared to the control group. The percentage of lymphocytes increased significantly ($P \geq 0.05$) in the blood of animals treated with the nano-zinc, methionine, and lysine mixture compared to the control group after 120 days of treatment. The percentage of monocytes and granulocytes decreased significantly in the sixth and eighth treatments compared to the control group. Total blood proteins also showed a significant increase ($P \geq 0.05$) in animals in the sixth, seventh, and eighth treatments compared to the control group, while serum albumin levels did not significantly differ between the different treatments. The results also showed a significant increase ($P \geq 0.05$) in the serum globulin levels in animals in the eighth treatment compared to the control group after 60 and 120 days of treatment .

Keywords: nano zinc, protected amino acids, methionine, lysine, some hematological parameters.

Introduction

Sheep are the most important and oldest species of domesticated livestock. The global

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sheep population currently exceeds one billion heads, 19% of which are found in Asia and Africa (Yousif, 2023). The livestock sector faces rising feed and raw material prices, which requires alternative sources with better bioavailability and high efficiency. Nanotechnology offers advantages for using nanoscale minerals in livestock feed, in addition to their benefits for human and animal health. Trace elements are essential, although they are required in small quantities (less than 100 mg/kg dry matter). They are essential for maintaining animal health and contributing to growth and reproduction. Mineral scarcity in feed significantly impacts animal health and production performance (Khalid, 2023). The bioavailability of some free minerals in feed is low and does not meet the needs of an animal (Princewill et al., 2015). Trace minerals present in feed are not sufficiently available to animals due to their interactions with each other and their association with certain chemical compounds such as lectins, phytates, and oxalates, which prevent their absorption and lead to deficiency in the body (Hummel et al., 2020). Because microbial protein and dietary protein do not meet the needs of specific amino acids, Such as methionine and lysine (Abdel-Ghani et al. 2011). These are essential amino acids for protein synthesis, optimal growth, and high production efficiency. Studies have indicated that methionine and lysine are essential for protein synthesis necessary for growth and production (El-Tahawy et al., 2013). Protected amino acids also improve carcass characteristics and meat quality by providing nutritional value and a distinctive flavor (Al-Jebory, 2017). Therefore, the current study aimed to investigate the biological effects of adding nano-zinc and protected amino acids (lysine and methionine), individually, and in combination, on some blood profile variables and serum proteins in Awassi lambs.

Materials and Methods

Thirty-two Awassi lambs, aged 5-7 months, with an average weight of 26.56 ± 0.20 kg, were used and randomly distributed into eight treatments, four lambs per treatment.

.1-Treatment 1: The control group was considered and fed. Their animals were fed a basal diet without any additives.

.2-Treatment 2: The animals were fed the basal diet with an oral dose of the amino acid methionine at 2 g/head per day.

.3-Treatment 3: The animals were fed the basal diet with an oral dose of the amino acid lysine at 4 g/head per day.

.4-Treatment 4: The animals were fed the basal diet with a mixture of the amino acids methionine and lysine at 2 and 4 g/head per day, respectively.

.5-Treatment 5: The animals were fed the basal diet with an oral dose of nano-zinc at 15 mg/head per day.

.6-Treatment 6: The animals were fed the basal diet with an oral dose of the amino acid methionine at 2 g/head per day plus nano-zinc (15 mg/head per day.(

-7Treatment Seven: The animals were fed the basal diet with an oral dose of the amino acid lysine (4 g/head per day) plus 15 mg/head per day of nano zinc.

-8Treatment Eight: The animals were fed the basal diet with an oral dose of a mixture consisting of the amino acids methionine and lysine (2 and 4 g/head per day, respectively) plus nano zinc (15 mg/head per day.(

Experimental Animal Management

The experimental animals were subjected to a 14-day pre-treatment period during which they were hygienically cared for before the start of the experiment to protect them from potential pathological infections. The animals were fed

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a concentrated feed (Erbil Feed Company) as shown in Table (1) at 3% of live body weight per day, individually for each treatment, and at 3% of live weight, divided into two meals, morning and evening. The feed quantity was adjusted every two weeks based on weight changes, with roughage (hay) freely available. Clean water was provided in the pen for each group, and mineral salt cubes were placed inside each pen for all groups. Blood samples were collected regularly every forty-five days at 7:30 a.m. after the animals were off feed for 12 hours, from the jugular vein in the neck area using a 10 ml wine syringe into clean, sterile wine plastic tubes.

Table (1): Effect of treatment with nano-zinc and protected amino acids (lysine and methionine) on some physical blood traits in Awassi lambs (mean ± standard error(

treatments	Physical blood traits		
	Red blood cells /10^6×)µL(	Hemoglobin)g/dL(	Packed blood cells (%)
After 60 days of treatment			
T1	0.01 ± 10.14b	0.55 ± 11.23b	0.21 ± 30.88b
T2	0.31 ± 10.72ab	0.45 ± 11.63ab	0.65 ± 32.10ab
T3	0.48 ± 11.39ab	0.14 ± 11.36b	0.42 ± 31.96ab
T4	0.46 ± 11.38ab	0.59 ± 12.33ab	0.65 ± 32.50ab
T5	0.88 ± 11.48ab	0.70 ± 11.83ab	0.35 ± 32.00ab
T6	0.47 ± 11.39ab	0.29 ± 12.03ab	0.33 ± 31.90ab
T7	0.77 ± 12.01ab	0.33 ± 13.16a	0.84 ± 32.70ab
T8	0.91 ± 12.28a	0.68 ± 12.73ab	0.82 ± 33.48a
significant	*	*	*
After 120 days of treatment			
T1	0.55 ± 11.04b	0.35 ± 11.50b	0.44 ± 31.43b
T2	0.31 ± 11.17b	0.22 ± 11.86b	0.30 ± 31.80b
T3	0.53 ± 11.11b	0.34 ± 11.93b	0.45 ± 32.03b
T4	0.50 ± 12.20ab	0.21 ± 11.70b	0.28 ± 31.80b
T5	0.54 ± 11.67ab	0.37 ± 11.80b	0.28 ± 33.63a
T6	1.23 ± 12.49ab	0.20 ± 12.86ab	0.73 ± 33.60a
T7	0.62 ± 12.73ab	0.79 ± 12.33ab	0.63 ± 33.66a
T8	0.76 ± 13.66a	0.46 ± 13.33a	0.16 ± 34.73a
significant	*	*	*

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:*Indicates significant differences between the means at a probability level of (p≤0.05(
T1: Control treatment T2: Treatment with the amino acid methionine (2 g/head/day) T3: Treatment with the amino acid lysine (4 g/head/day) T4: Treatment with the amino acids methionine and lysine (2 and 4 g/head/day, respectively) T5: Treatment with nano zinc (15 mg/head/day) T6: Treatment with the amino acid methionine and nano zinc (2 g and 15 mg/head/day, respectively) T7: Treatment with the amino acid lysine and nano zinc (4 g and 15 mg/head/day, respectively) T8: Treatment with the amino acids methionine, lysine and nano zinc (2 g, 4 g and 15 mg/head/day) (a day in a row.(

:2Effect of treatment with nano-zinc and protected amino acids (methionine and lysine) on white blood cells and differential counts in Awassi lambs.

The results of Table (2) showed a significant decrease ($P \leq 0.05$) in the number of white blood cells in the blood of animals in the eighth treatment compared to the rest of the treatments, except for treatments six and seven, which did not differ significantly from treatment eight after 60 days of treatment. Significant differences were also observed in the number of lymphocytes, as treatment eight recorded a significant increase ($P \leq 0.05$) in the number of lymphocytes compared to the control and third treatments, while they did not differ significantly from the rest of the treatments. As for mononuclear cells, no significant differences were observed between the different treatments. All treatments also recorded a significant decrease ($P \leq 0.05$) in the percentage of granulocytes in the blood of treated animals compared to the control treatment, except for treatment three, after 60 days of treatment. To track the biological effect of treatment with nano zinc and protected amino acids (methionine, lysine) after 120 days of treatment, the animals treated in the eighth, sixth, fifth, seventh and fourth treatments showed a significant decrease ($P \geq 0.05$) in the number of total white blood cells compared to the first treatment (control) and the second, while it did not differ significantly from the third treatment. From the same table, it is noted that the percentage of lymphocytes witnessed a significant increase ($P \geq 0.05$) in the eighth and sixth treatments compared to the rest of the treatments. The second, third, fourth and seventh treatments also recorded a significant increase ($P \geq 0.05$) compared to the control group, while it did not differ significantly from the fifth treatment. As for the percentage of monocytes, their percentage decreased significantly ($P \geq 0.05$) in the blood of animals treated in the sixth and eighth treatments compared to the control group, while the rest of the treatments did not differ significantly from the control group. As for granulocytes, their percentages showed a significant decrease ($P < 0.05$) in the blood of animals in the eighth and sixth treatments compared to the first, fourth, and fifth treatments, while the remaining treatments did not differ significantly from the control group.

The significant decrease in the total white blood cell count may be due to zinc's ability to enhance antioxidants (increase glutathione and decrease malondialdehyde) in the body, as well as its anti-inflammatory role .

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Table (2): Effect of treatment with nano-zinc and protected amino acids (lysine and methionine) on the number and differentiation of white blood cells in Awassi lambs (mean ± standard error.(

treatments	white blood cells			
	White blood cells ($\times 10^3$/µL)	Lymphocytes(%)	Monocytes(%)	Granulocytes(%)
After 60 days of treatment				
T1	0.61 ± 8.61a	2.19 ± 59.00c	1.55 ± 10.50 a	1.31 ± 30.25a
T2	0.62 ± 8.65a	1.89 ± 63.50abc	1.03 ± 12.75a	0.95 ± 23.50bcd
T3	0.46 ± 6.31b	2.32 ± 59.50bc	1.47 ± 13.00a	1.03 ± 27.25ab
T4	0.72 ± 7.00ab	2.28 ± 65.25abc	1.10 ± 10.75a	1.47 ± 24.00bcd
T5	0.58 ± 6.85ab	2.39 ± 62.25abc	1.10 ± 11.75a	1.47 ± 26.00bc
T6	0.50 ± 5.36bc	0.40 ± 66.00ab	1.37 ± 13.25a	1.47 ± 21.00de
T7	0.77 ± 5.46bc	1.88 ± 66.25ab	1.32 ± 10.50a	0.62 ± 23.25cd
T8	0.61 ± 4.20c	2.44 ± 67.00a	1.60 ± 13.75a	1.29 ± 19.00e
significant	*	*	N.S	*
After 120 days of treatment				
T1	0.57 ± 9.98a	1.22 ± 60.00c	1.88 ± 11.25a	0.86 ± 28.50a
T2	0.27 ± 9.91a	2.56 ± 67.25b	1.04 ± 7.50ab	1.93 ± 25.25 ab
T3	0.25 ± 8.40ab	1.65 ± 66.25b	0.64 ± 8.50ab	1.84 ± 25.50ab
T4	0.95 ± 7.31bc	1.19 ± 67.50b	0.95 ± 7.50ab	1.65 ± 25.75a
T5	0.57 ± 6.70bc	1.65 ± 64.75bc	1.93 ± 7.75ab	0.62 ± 27.25a
T6	0.62 ± 6.65bc	0.47 ± 75.25a	0.28 ± 3.50c	0.47 ± 21.25bc
T7	0.81 ± 6.83bc	3.11 ± 67.25b	1.32 ± 7.50ab	1.84 ± 25.25ab
T8	0.33 ± 5.70c	0.25 ± 76.25a	0.64 ± 5.50bc	0.62 ± 18.25c
significant	*	*	*	*

:*Indicates significant differences between means at a probability level of ($p \leq 0.05$(

NS: Indicates no significant differences between means

T1: Control treatment. T2: Treatment with the amino acid methionine (2 g/head/day). T3: Treatment with the amino acid lysine (4 g/head/day). T4: Treatment with the amino acids methionine and lysine (2 and 4 g/head/day, respectively). T5: Treatment with nano zinc (15 mg/head/day). T6: Treatment with the amino acid methionine and nano zinc (2 g and 15 mg/head/day, respectively). T7: Treatment with the amino acid lysine and nano zinc (4 g and 15 mg/head/day, respectively).

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T8: Treatment with the amino acids methionine, lysine, and nano zinc (2 1 g, 4 g, and 15 mg/head/day, respectively.

This may indicate that it provides protection to blood cells from oxidative damage during their formation or after they are produced from the bone marrow and directed to perform their vital functions in the body (Abdel-Fattah et al., 2011). Zinc stimulates the production of the protein metallothionein, an effective scavenger of hydroxyl radicals, providing protection against immune-mediated free radical attack (phagocytosis) (Thompson, 2022). As for the significant increase in the percentage of white blood cells and lymphocytes in the treatment animals compared to the control group, it may be due to the synergistic effect of zinc and protected amino acids, as zinc plays an important role in activating the immune system through the development of the thymus gland. and bone marrow (Goswami et al., 2005). Zinc plays an important role in the bioactivation of thymulin, a hormone produced by the thymus gland. It binds to its high-affinity receptors on T lymphocytes, stimulating the division, proliferation, and function of T lymphocytes in various lymphoid tissues throughout the body (Prasad, 2008). Zinc also has an antioxidant role, protecting cells from the toxic effects of free radicals. It is also an element that contributes to increased absorption of minerals important for maintaining the vital processes of body cells, including white blood cells (Sanchez et al., 2009). The significant decrease in the percentage of granular white blood cells is consistent with the findings of Manimaran et al. (2022), who demonstrated that adding copper sulfate and zinc oxide to the diet of Osmanabadi goats at concentrations of 100 mg/goat/day and 40 mg/goat/day, respectively, led to a significant decrease in the percentage of neutrophils, while no significant effect was found in the percentage of basophils. This result is consistent with the findings of Abd-Eldaim et al. (2018), Shareef et al. (2019), Effah-Yaboah et al. (2021), Abdelgayed et al. (2022), Yusuf et al. (2023), and Al-Abbasi (2023.(

:3Effect of treatment with nano zinc and protected amino acids (methionine and lysine) on serum proteins of Awassi lambs .

The results of the statistical analysis in Table (3) showed a significant effect of treatment with nano zinc and protected amino acids (methionine and lysine) on the level of total proteins in the serum of local lambs. The eighth treatment recorded a significantly excelled ($P \leq 0.05$) in the value of this trait compared to the control group and the second, fourth, and fifth treatments, while it did not differ significantly from the rest of the treatments. The sixth and seventh treatments also recorded a significant increase ($P \leq 0.05$) compared to the control group after 60 days of treatment. From the same Table (3), it is noted that the level of serum albumin was not significantly affected by the various treatments after 60 days of treatment. In the same context, animals in the eighth treatment showed a significant increase ($P \leq 0.05$) in serum globulin levels compared to the rest of the treatments, except for the seventh treatment, which did not significantly differ from them. To track the biological effect of treatment with nano zinc and protected amino acids on the level of total proteins in the blood serum after 120 days of treatment, the level witnessed a significant increase ($P \leq 0.05$) in the eighth treatment compared to the rest of the treatments, except for the sixth and seventh treatments, which did not significantly differ from them. Meanwhile, albumin levels

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did not record any significant differences between the different treatments. From the same Table (3), it is noted that the level of globulin in the blood serum of animals in the sixth and eighth treatments increased significantly ($P \leq 0.05$) compared to the control, second, and third treatments, while it did not significantly differ from the remaining treatments. The improvement in total protein and globulin values in treated animals compared to the control group may be due to the role of zinc and the protected amino acids (methionine-lysine) in improving antioxidants, liver function, and enhancing the immune status of treated animals. The significant increase in total blood protein with zinc supplementation may indicate increased protein synthesis resulting from increased secretion of anabolic hormones responsible for the utilization of amino acids such as growth hormone and insulin-like growth factor-1 (Adriani and Wirjatmadi, 2014). Total blood protein levels are closely related to the body's nutritional status; therefore, the improvement in protein metabolism may be attributed to the added amino acids. Improvement in the amino acid balance in protein metabolism occurs when amino acids, especially the methionine-lysine mixture, are added to increase total blood protein concentration (Movaliya, 2013). Protected protein reduces its degradation rate in the rumen and increases its access to the stomach and small intestine, leading to increased amino acid absorption and, consequently, increased serum protein concentration (Richardel, 2004). Our current findings are consistent with the findings of studies by Shaawi et al. (2017), Elwakeel et al. (2018), Abo Elhaded et al. (2021), Al-Zubaedi et al. (2021), Kumar et al. (2021), Abdelgayed et al. (2022), Yusuf et al. (2022), Yusuf et al. (2023), Al-Abbasi et al. (2023), and Soliman et al. (2024.(

Table (3): Effect of treatment with nano zinc and protected amino acids (lysine and methionine) on blood serum proteins in Awassi lambs (mean ± standard error.(

treatments	Serum proteins (g/dL)		
	Total proteins	**Albumin**	**Globulin**
After 60 days of treatment			
T1	0.28 ± 5.85c	0.47 ± 3.25a	0.26 ± 2.60b
T2	0.61 ± 6.40bc	0.47 ± 3.25a	0.41 ± 3.15b
T3	0.79 ± 7.50abc	0.47 ± 4.25a	0.55 ± 3.25b
T4	0.89 ± 6.50bc	0.95 ± 3.50a	0.34 ± 3.00b
T5	0.50 ± 6.50bc	0.40 ± 3.00a	0.28 ± 3.50b
T6	0.58 ± 8.05ab	0.47 ± 4.75a	0.66 ± 3.30b
T7	0.82 ± 8.02ab	0.25 ± 4.25a	0.64 ± 3.77ab
T8	0.40 ± 9.00a	0.70 ± 4.00a	0.54 ± 5.00a

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significant	*	N.S	*
After 120 days of treatment			
T1	0.20 ± 6.10c	0.55 ± 3.62a	0.37 ± 2.47c
T2	0.58 ± 7.05bc	0.64 ± 4.50a	0.09 ± 2.55c
T3	0.35 ± 7.32bc	0.47 ± 4.62a	0.27 ± 2.70bc
T4	0.38 ± 6.95bc	0.47 ± 3.75a	0.44 ± 3.20abc
T5	0.25 ± 6.95bc	0.40 ± 3.00a	0.25 ± 3.95ab
T6	0.23 ± 8.10ab	0.62 ± 3.75a	0.83 ± 4.35a
T7	0.46 ± 7.72ab	0.40 ± 4.00a	0.48 ± 3.72abc
T8	0.51 ± 8.70a	0.47 ± 4.25a	0.12 ± 4.45a
significant	*	N.S	*

:*Indicates significant differences between means at a probability level of ($p \leq 0.05$(

NS: Indicates no significant differences between means

T1: Control treatment. T2: Treatment with the amino acid methionine (2 g/head/day). T3: Treatment with the amino acid lysine (4 g/head/day). T4: Treatment with the amino acids methionine and lysine (2 and 4 g/head/day, respectively). T5: Treatment with nano zinc (15 mg/head/day). T6: Treatment with the amino acid methionine and nano zinc (2 g and 15 mg/head/day, respectively). T7: Treatment with the amino acid lysine and nano zinc (4 g and 15 mg/head/day, respectively). T8: Treatment with the amino acids methionine, lysine, and nano zinc (2 15 mg/head/day, 4 g, and 15 mg/head/day, respectively.

Conclusions

The results of this study showed that adding nano-zinc with protected amino acids (methionine and lysine) had a positive effect on blood physiological parameters, including hemoglobin, packed cell volume, and white blood cell count in general and lymphocyte count in particular. Improvement in total blood protein and globulin levels was also demonstrated, thus positively impacting the studied physiological parameters.

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