Mesopotamia Journal of Agriculture

https://magrj.mosuljournals.com

IMPACT OF ZINC SUPPLEMENTATION ON NUTRIENTS DIGESTIBILITY AND BLOOD MINERALS CONCENTRATION DURING HOT SEASON OF LOCAL GROWING LAMBS

Araz Q. S. Abbi
Department of Animal Production, College of Agricultural Engineering Sciences, University of Duhok, Duhok, Iraq

Article information
Article history:
Received: 29/01/2024
Accepted: 02/03/2024
Published: 31/03/2024

Keywords:
Blood biochemical, digestibility coefficient, heat stress, Serum minerals, Zinc.

DOI:
https://doi.org/10.33899/mja.2024.146430.1364

Correspondence Email:
araz.qasim@uod.ac

ABSTRACT

The current experiment was carried out to determine the effect of zinc (Zn) supplementation on digestibility of nutrients and blood minerals concentration during hot season of local growing lambs. In the current experiment, twenty-seven local growing Awassi lambs with live weight 25.92 kg± SD=4.29, and aged 8 months ±SD=1.1, were allocated randomly in single pens (1.50 x 1.30 m) by live weight in indoor hall to one of three experimental treatments during July (hot season). Zinc was added to drinking water in three different levels, 0 ml/day (0 mg Zn), 3 ml/ day (36 mg Zn) and 6 ml/day (72 mg) as treatments one (T1), two (T2) and three (T3) respectively. The animals were adapted to the experimental treatments for 3 weeks, followed by 5 days of feed and fecal samples collection for further laboratory proximate analysis to determine the digestibility coefficient of nutrients. At day three of sample collection, blood samples were collected by jugular venipuncture at 11 a.m. for blood minerals and biochemical determination. The results showed that Zn supplementation increased ($P<0.001$) blood serum Zn concentration. In addition, Zn supplementation decreased both blood serum albumin ($P=0.001$) and glucose ($P=0.010$) in T3, whereas serum ALP concentration increased ($P=0.042$) in T3. Zinc supplementation effectively improved nutrient digestibility coefficient, increased NDF ($P=0.004$) and ADF ($P=0.020$) digestibility, and tended to increase DM digestibility. The above results indicate that Zn supplementation may have a role in decreasing the negative effect of heat stress.

INTRODUCTION

Climate change, including an increase in temperature, is one of the concerns facing many countries around the world, and Iraq is more severely suffering from temperature increase than many countries (Nosir, 2023). Farm animals are affected by the ambient temperatures, which influence on heat balance, feed intake and metabolism (Fuquay,1981). Furthermore, heat stress can change the dynamic characteristics of the digestion processes that affect animals' nutrition(Beede and Collier,1986). In ruminants, the documented results are inconsistent regarding to the impact of heat stress on nutrient digestibility. Some authors documented that the hot environments increased diet digestibility in cattle (Miaron *et al.*,1992 and NRC,1981). Others reported that the hot temperature had no impact or decreased the digestibility of nutrients in small ruminants (Silanikove,1992), and dairy cattle (Mathers *et al.*,1989 and NRC,1981). For instance, Yadav *et al.*, (2016) found that the digestibility of organic matter (OM) and acid detergent fiber (ADF) decreased at

40°C in crossbred cattle. In addition, Bernabucci *et al*., (2009) documented that heat stress had decreased dry matter (DM) intake and digestibility, and the digestibility coefficient of neutral detergent fiber (NDF), ADF, OM and non-structural carbohydrates in sheep. This means that the hot ambient temperatures may have a negative role in diet digestibility of growing lambs.

The negative effect of environmental stress (heat stress) can be reduced via supplementation of antioxidant nutrient including vitamins A, C and E; and antioxidant minerals (chromium and Zn)(Sunil Kumar *et al*.,2011 and Rahawi *et al*.,2022). Blood content of minerals has a direct effect on the activity of these antioxidant enzymes (Song and Shen,2020). The metalloenzymes antioxidants including superoxide dismutase (Zn and Cu), glutathione peroxidase (Se) and catalase (Iron) play an important role in protection of intracellular components against oxidative damage(Abbi,2020; McDowell *et al*.,2007 and Spears and Weiss,2008). Therefore, the oxidative stress of growing lambs might reduce via supplementation of Zn as antioxidant (Song and Shen,2020 and Abdul-Majeed *et al.*,2022).

Furthermore, studies have documented that Zn supplementation improved nutrients digestibility. For example, Alimohamady *et al*., (2019) documented that supplementation of organic Zn improved the digestibility of OM, CP (crude protein) and ADF in lambs. Similarly, Garg *et al*., (2008) found that organic Zn supplementation improved both ADF and cellulose digestibility. Hassan *et al*., (2016) also found that supplementation of Zn froorganic and non-organic sources improved nutrient digestibility, including crude fiber (CF), DM, OM, CP, fat and total digestible nutrients in growing buffalo calves. In addition, Zn supplementation increased rumination and feeding duration under heat stress condition (Abbi *et al*.,2022).

As discussed above, heat stress has a negative effect on nutrient digestibility in ruminants; while, Zn supplementation can reduce heat stress and improve nutrient digestibility. However, the role of Zn supplementation during heat stress in nutrients digestibility is poorly understood. Therefore, the current experiment objective is to determine the impact of Zn supplementation on nutrient digestibility and blood mineral concentration during hot season in local growing lambs.

MATERIALS AND METHODS

Ethical approval

Ethic approval (Authority No: AEC 19062021) was obtained from the local Animal Ethic Committee, Animal Research Authority and Approval for Animal Experimentation, Animal Production Department, College of Agricultural Engineering Sciences, University of Duhok, Iraq.

Experimental design

The current research was carried out at Animals Farm Project of the department of Animal Production, College of Agricultural Engineering sciences, University of Duhok during summer season (heat stress season). Initially, twenty-seven locals growing Awassi lambs with live weight 25.92 kg± SD=4.29, and aged 8 months ±SD= 1.1 were allocated randomly in single pens (1.50 x 1.30 m) by live weight in indoor hall to one of three experimental treatments as treatments one (T1), two (T2) and three (T3) respectively. The Zn (Zinc sulphate, monohydrate (12mg/ml

(ACTIVE BIOTIN Zn)) was supplemented in three different levels to fresh drinking water, 0 ml/day (0 mg Zn), 3 ml/ day (36 mg Zn) and 6 ml/day (72 mg) as treatment one (T1), treatments two (T2) and three (T3) respectively.

Experimental Routine

The lambs were daily fed on concentrate (400g) and hay in two meals at 9:00 morning and 17:00 evening in individual clean plastic buckets, then the quantity of feeding meal gradually increased (10%) to provide *ad-libitum* with the beginning of adaptation week (week one). The experimental diet was formulated as isonitrogenous and isoenergetic to meet the requirements of growing lambs according to AFRC (1993). Blood samples were obtained at samples collection week (day three) by jugular venipuncture at 11 a.m. (10 ml) for each lamb using a 20 G needles into tubes (anticoagulant-free test tube), then spin for 15 minutes (3000 rpm). The separated blood serum stored at -20 ºC in Eppendorf analysis of blood minerals, total protein (TP), albumin, urea, glucose, aspartate amino transferase (AST, alanine amino transferase (ALT), alkaline phosphate (ALP) and creatine kinase (CK) for further laboratory analysis.

The adaptation of lambs to experimental treatments took three weeks followed by 5 days fecal collection. During week 4 approximately 50 g of fecal grab together with feed samples were collected from lambs over 5 days at 11:00 a.m. on each treatment to estimate indirect diet digestibility using acid insoluble ash as internal marker (Block *et al*.,1981). Throughout the fecal collection, feed intake was also recorded. Then the collected feed and fecal samples were oven dried (20 g), bulked and milled through a 1 mm screen.

Chemical analysis

The proximate analysis of feed and fecal samples was measured according to Association of Official Analytical Chemists (AOAC,2016). Dry matter (DM) measured according to method of AOAC, (2016) 930.15. Ash was measured by method AOAC, (2016) 942.05, then OM measured by calculating the difference between sample weight and ash weight. The method of CF determination was AOAC, 2016 (962.09). Crude protein was determined by method of AOAC, 2016 (968.06) by using an auto Dumatherm analyzer (Dumatherm N., Gerhard apparatus) (Sweeney,1989). Near-infrared reflectance spectroscopy (NIRS) instrument (Perten, DA 7250) both NDF and ADF. Blood serum metabolites were measured using auto biochemistry analyzer from Fujifilm apparatus (DRI-CHEM NX500i, China) for total protein total (using TP-P FUJI DRI-CHEM SLIDE), albumin (using ALB-P FUJI DRI-CHEM SLIDE), urea (using BUN-P FUJI DRI-CHEM SLIDE), glucose (GLU-P FUJI DRI-CHEM SLIDE), Aspartate amino transferase (AST) (using COT/AST-P FUJI DRI-CHEM SLIDE), Alanine amino transferase (ALT)(using GPT/ALT-P FUJI DRI CHEM SLIDE), Alkaline phosphate (ALP)(using ALP-P FUJI DRI-CHEM SLIDE) and Creatine kinase (CPK)(using CPK-P FUJI DRI-CHEM SLIDE). Blood Minerals including Zn (zinc), Ca (calcium), P (phosphorus), Cu(copper), Fe (iron), Mn (manganese), S (sulfur), Pb (lead), Se (selenium) and Mo (molybdenum) were determined using inductively coupled plasma mass spectrometry (ICP-MS) instrument.

Nutrient digestibility

Dry matter digestibility was indirectly estimated using acid insoluble ash in lambs using method of Van Keulen and Young (1977) by using the bellow equations:

$$\%AIA = \left[\frac{\text{crucile weight (g)+ash}-\text{ crucibleweight (g)}}{\text{dry sapmle weight (g)}}\right] \times 100$$

Digestibility of feed DM was also calculated as:

$$\text{Feed DM Digestibility (kg/kg)} = 1 - 1 \times \frac{\text{(g/kg DM AIA in feed)}}{\text{(g/kg AIA in fecal)}}$$

The fecal DM output (g/d) for each lamb was also calculated as:
Fecal DM output (g/d) = DMO (kg) – (DMI (kg) × digestibility (kg/kg))
The digestibility of OM, CP (crude protein), CF (crude fiber), NDF and ADF were also calculated from DMI as bellow:

$$\text{Nutrient digestibility (g/d)} = \left[\frac{\text{Nutrient intake (g/d) – Nutrient output (g/d)}}{\text{Nutrient intake (g/d)}}\right]$$

Statistical analysis

Statistical software package (Genstat 14th edition, VSN International Ltd,UK) were used for statistical analysis. All of the data were normally distributed then analyzed by one way analysis of variance (ANOVA). A various group of all parameters were compared using Tukey test. The effect at P<0.05 was reported a significant, P between <0.01 and <0.05 was reported a trend.

RESULTS AND DISCUSSION

Blood minerals concentration

Zinc supplementation had an effect (P<0.05) on blood Zn concentration Table (1), Zn supplementation increased (P<0.001) blood serum Zn concentration in T2 and T3 treatments respectively. Zinc supplementation had no effect on the blood concentration of other minerals Table (1). Zinc level in the blood in sheep is 80 to 120 µg/dl (NRC,2005 and NRC,2007). Zinc supplementation in the current experiment was effective and increased blood plasma concentration of Zn in both T2 and T3 treatments respectively (48.4 and 54.6 µg/dl), however, was less than normal range of Zn concentration in sheep blood according to both NRC (2005) and NRC (2007) In addition, the current findings were less than the previous published experiments. Minervino *et al*., (2018) documented that sheep blood Zn concentration ranged between 116 to 272 µg/dl in supplemented (300 mg Zn/kg DM) and non-supplemented treatments respectively. In addition, the results of current experiment disagreed those published by Aliarabi *et al*., (2015) who documented that the concentration of lambs blood plasma Zn ranged between 93 to 147 µg Zn/dl with Zn supplementation (0 to 40 mg Zn/kg DM Zn supplementation). Chavan *et al*., (2021) found that kids Zn level in kids blood plasma ranged between 83 to 216 µg Zn/dl in non-supplemented and supplemented (40 mg Zn /kg DM) treatments respectively.

Table (1): Lambs blood minerals content supplemented with different levels of zinc

	T1	T2	T3	SED	P-value
Zn (µg/dl)	41.2a	48.4b	54.6c	1.190	<0.001
Fe (µg/dl)	144	142	132	14.40	0.266
Ca (mg/dl)	12.0	10.0	12.0	1.010	0.114
S (µg/dl)	84.3	84.0	83.1	2.640	0.897
Pb (mg/l)	2.23	2.34	2.30	0.265	0.922
Cu (µg/dl)	52.4	46.6	51.3	6.150	0.606
Se (µg/dl)	0.25	0.30	0.30	0.041	0.446
Mn (µg/dl)	1.10	1.10	1.00	0.11	0.359
Mo (µg/dl)	0.05	0.06	0.06	0.006	0.183
P (mg/dl)	6.30	6.50	5.40	0.81	0.340

T1= 0 mg Zn/day (treatment one), T2= 36 mg Zn/day (treatment two), T3= 72 mg Zn/day (treatment three). Means with different superscript letters differ (P<0.05). SED= standard error of deviation.

Many stressors have effect on the ruminants during the production including exposure to pathogens, transportation and weaning (VanValin *et al*.,2020). Heat stress considered as a factor of environment that contribute to oxidative stress, this leads to a heightened generation of reactive oxygen species (ROS) and free radicals (FR), coupled with antioxidant reduction that normally mitigate the negative effects of oxidative stress (Ahmed and Abdul-Rahman,2023 and Saker *et al*.,2004). The concentration of minerals in the blood is highly related to the activity of antioxidants (Song and Shen,2020). Too high or too low of blood minerals will decrease the antioxidant enzymes activity (Song and Shen,2020). During a stressful condition, ruminants might experience an increased requirement for Zn because of its crucial role in essential defense mechanisms such as oxidative stress (VanValin *et al*.,2020). Deficiency of Zn can increase the cell membranes oxidative damage by free radicals (Kaki *et al*.,2020). Zinc works as an antioxidant by several mechanisms. The first mechanism is the competition between copper and iron with Zn, and ions to bind with proteins and cell membranes, separating these redox active minerals to catalyze OH production from H_2O_2. Secondly, binding of both sulfhydryl groups of biomolecules and Zn to protect them from oxidation. Finally, Zn role in the increasing molecules activation, antioxidant proteins, enzymes such as catalase, and SOD (Bao *et al*.,2014). The acute stress may reduce plasma concentration of Zn, this could be due to an increase of metallothionein in response to stress and increase fecal Zn excretion (VanValin *et al*.,2020). Yadav *et al*., (2016) documented that the heat stress of experimental cattle started at 35 ºC exposure, however the intense heat stress recorded when the cattle exposed at 40 °C. The environmental temperature that recorded during the current experiment was above 40°C. This means that the lambs in the current experiment were under heat stress, this could be the reason that the blood plasma Zn concentration in the current experiment was low comparing to the other published experiments. This may need further work to refine the needs of ruminants to minerals supplementation during hot climate season.

Blood metabolites

Zinc supplementation had effect on blood metabolites Table (2), Zn supplementation decreased blood glucose (P=0.01) and albumin (P=0.001) concentration in T3 treatment. In addition, Zn supplementation increased (P=0.042) blood serum ALP in T2 treatment. Zinc supplementation had no effect on the other blood metabolites that presented in Table (2). Supplementation of Zn in higher levels leads to increases in Alkaline phosphatase (ALP) activities. The current results similar to the finding of Liu *et al*., (2015) who found that supplementation of goats with Zn (45.9 mg Zn/kg) increased the activity of ALP among different supplementation groups (20, 40 or 80 mg Zn/kg). Similarly, Kumar *et al*., (2006) recorded an increase in the activity of bull ALP supplemented a dietary Zn (32.5 mg Zn/kg) in treatments contained 35 or 70 mg Zn/kg. However, Aliarabi *et al*., (2015) reported Zn supplementation from different sources had no significant effect on the level of ALP in growing lambs.

Zinc is cofactor of many enzymes, the activities of these enzymes are indicators of the body Zn status (Suttle NF,2010). Alkaline phosphatase (ALP) is an enzyme that relies on Zn and plays a role in calcium absorption, and growing animals' development (Alimohamady *et al*.,2019). Alkaline phosphatase (ALP) plays a fundamental physiological role in cell division and growth regulation (Aliarabi *et al*.,2015). In growing animals, the metabolically active tissues have a high concentration of ALP (Aliarabi *et al*.,2015). Cho *et al*., (2007) documented a reduction in ALP activity in animals deficient in Zn. Aliarabi *et al*., (2015) reported Zn level in basal diet of lambs about 22.47 mg/kg DM is not enough for adequate activity of ALP, while ALP activity can be improved by 40 mg/ kg DM Zn supplementation as $ZnSO_4$. The reduction of heat stress can be indicated by increases of ALP (Nasrollahi *et al*.,2019). Therefore, The ALP increases in Zn supplemented treatment in the current experiment may be due to the reduction of heat stress by Zn supplementation.

Mandal *et al*., (2008) reported that the activities of ALT and AST were similar between crossbred calves supplemented with a same level of Zn from different sources (35 mg Zn/kg DM) comparing to control treatment supplemented with 32.5 mg/kg Zn. Alimohamady *et al*., (2019) indicated that supplementation of 19.72 mg Zn/kg DM was not adequate for tissue lesion inhibition. The muscle and liver lesions can be assessed by a routine measuring of the blood activities of AST, ALT, and CPK (Alimohamady *et al*.,2013). The blood concentration of AST, ALT, and CPK was similar between treatment groups in the current experiment, this means that there was no muscle or liver lesions in the current experiment.

Previous published studies documented that blood glucose reduced by heat stress (Abeni et al.,2007 and Wheelock et al.,2010). Attia (2016) found a reduction in blood glucose concentrations under high ambient temperature as a result of higher insulin activity (Baumgard and Rhoads, 2013 and Rhoads *et al*.,2009), or feed intake reduction (Rhoads *et al*.,2009). Omar and Barwary (2022) documented that Zn supplementation (36 mg Zn/day) kept blood glucose level from reduction or increased blood glucose concentration (only in one week) in lambs over six weeks of experiment. The current experiment results agree with the above published works and Zn supplementation kept blood glucose from reduction in T2 treatment. However, in T3 treatment blood glucose reduced, the lower DM intake (about 13%) could be the reason of the lower glucose concentration in T3 treatment (Rhoads *et al*.,2009).

Table (2): Lambs blood metabolites supplemented with different levels of zinc

	T1	T2	T3	SED	P-value
Total protein (g/dl)	6.41	6.35	6.06	0.319	0.516
Albumin (g/dl)	2.97b	2.96b	2.58a	0.104	0.001
Urea (mg/dl)	20.2	18.7	18.3	2.820	0.781
Glucose (mg/dl)	67.3b	71.0b	59.5a	3.470	0.010
CPK (U/l)	80.0	76.2	70.2	8.480	0.524
AST (U/l)	105	107	119	17.38	0.671
ALT (U/l)	14.2	13.6	14.0	2.237	0.964
ALP (U/l)	209a	298b	197a	39.70	0.042

T1= 0 mg Zn/day (treatment one), T2= 36 mg Zn/day (treatment two), T3= 72 mg Zn/day (treatment three). Means with different superscript letters differ (P<0.05). SED= standard error of deviation.

Nutrient digestibility

Zinc supplementation had no effect on nutrients intake Table (3). Zinc supplementation reduced fecal output content of CF (P=0.031) in treatment T3 and NDF (P=0.014) in T2 and T3 treatments respectively Table (4). Zinc supplementation increased the digestibility coefficient of NDF (P=0.004) and ADF (P=0.020) in treatments T2 and T3 respectively; and tended to increase the DM digestibility in T2 and T3 treatments Table (5). Mallaki *et al*., (2015) recorded non-significant effect of organic Zn supplementation (20 mg Zn/kg DM) on OM and DM digestibility but increased NDF digestibility, these results agree with the current experiment however, the DM digestibility in the current experiment tended to be increased. Mallaki *et al*., (2015) suggested that Zn supplementation could have a positive effect on NDF digestibility. In addition, the results of current experiment also agree with Garg *et al*., (2008) who supplemented 20 mg/kg diet of organic Zn to a feedstuff contained 34 mg Zn/kg DM and recorded an improvement in ADF digestibility but there was no improvement in DM, NDF and CP digestibility which disagree with current experiment results in regard of NDF digestibility. In addition, the current findings agree with those published by Mandal *et al*., (2007) in term of DM and CP who recorded non-significant effect of Zn supplementation (35 mg Zn/kg DM) to a diet contained 32.5 mg Zn/kg DM on DM, CP, NDF and ADF digestibility in bulls, suggesting the reason of non-significant effect of Zn supplementation to that the basal diet Zn was met the ruminal microorganism requirements. Alimohamady *et al*., (2019) recorded non- significant effect of Zn supplementation (30 mg Zn/ DM) to a diet contained 19.72 mg Zn/kg DM on DM and NDF digestibility of growing lambs, while supplementation of zinc methionine and zinc proteinate (organic Zn) improved CP and ADF digestibility. In the current experiment, however the supplemented Zn was inorganic but the improvement of ADF digestibility is similar to the findings recorded by Alimohamady *et al*., (2019). Zinc supplementation may improve DM digestibility (DMD) in several ways. Zinc can improve the activity and growth of rumen microorganism, and activities of digestive enzymes (Suttle NF, 2010). Another way could be related hydrolase activity improvement of gastrointestinal tract by Zn supplementation (Hosseini-Vardanjani *et al*.,2020). This could be a reason that Zn supplementation improved digestibility coefficient in the current experiment.

Any kind of water restriction for about 2 hours should increase the digesta's retention time, which allow for more time for microbial production and degradation, and improve nutrient digestibility but the digestibility may reduce when the restriction prolongs to 3 hours (Ghassemi Nejad *et al*.,2014). In the current trial the Zn was supplemented to drinking water. All the lambs restricted from drinking water for 2 hours before adding Zn to drinking water to ensure that all the treatments (Zn) taken by the animals. This could be another reason that increased digestibility of both NDF and ADF. On the other hand, heat stress may affect on digestibility in many ways. Kadzere *et al*., (2002) documented that the digestibility can be increased by exposure to hot condition, this might be due to DMI reduction and feed retention time prolonging in the digesta. Rumen functions also can be affected by heat stress in several ways. Alert of rumen acid-base balance via the reduction flow rate of blood to epithelium of rumen, Saliva and its content of HCO_3 reduced by heat stress, this could impair rumen functions (Kadzere *et al*.,2002). In addition, Bernabucci *et al*., (2009) hypothesized that exposure of animals to a chronic heat stress leads to change in digestibility through increasing water intake, then the rumen contents dilute, reduce the activity of rumen bacteria, decrease the motility of rumen and finally reduce the production of saliva.

It has been reported that high ambient temperature had a positive digestibility of diet in Ayrshire cattle that exposed to 33ºC for 20 days, the reason could decrease in DMI (Mathers *et al*.,1989). Miaron *et al*., (1992), also found that housing of steers under warm condition increased the digestibility of DM and NDF comparing to others housed under thermoneutral conditions (28 ºC vs 10°C for 21 days). The slightly improvement of digestibility coefficients in animals might be due to passage rate reduction of digesta, and increases of retention time as a result of decrease motility of digestive system of animal that happens under hot climate condition (Bernabucci et al.,2009). In contrast, other researchers NRC (1981) documented that hot environments did not change or reduced diet digestibility of ruminants as a result of decreases blood flow rate to digestive tract tissues. On the other hand, Bernabucci *et al*., (1999) reported that exposure of Holstein heifers to hot environments for a short time improved diet digestibility, while the digestibility reduced when the exposure duration prolonged regardless of DM passage rate through the digesta. The results of a study conducted on ewes exposed to a chronic heat conducted by Bernabucci *et al*., (2009) recorded a reduction diet digestibility, pH and reduction in concentration of both amylolytic and cellulolytic bacteria, passage rate of digestive system decreases and lower osmolarity of rumen content, suggesting a significant dilution of rumen fluid and potential reduction of bacterial activity. The beneficial impacts of the DMI reduction and decreases of outflow rate of digestive system may have been outweighed by the negative effect of such a decline in the activity of bacteria in rumen on digestibility of diet (Bernabucci *et al*.,2009). Yadav *et al*., (2016) documented that the digestibility of DM, OM, NDF and ADF increased at 35 ºC comparing to 25 ºC, 30 and 40ºC as a result of increasing of mean retention time (MRT), rumen pH changes, temperature or bacterial population. Exposure of sheep to hot environment for short period of time (10 days) had no effect of DM, OM, NDF, and ADF digestibility coefficients but prolonging exposure to heat were reduced the digestibility (Bernabucci *et al*.,2009). As discussed in the previous section, the environmental temperature that recorded during the current experiment running was above 40°C. This means that the lambs in the current experiment were under heat stress. In this experiment, although there was Zn supplemented while the serum concentration of Zn was lower than previous published works. This means that Zn supplementation might have role in reducing of the negative effect of heat stress and improving nutrient digestibility.

Table (3): Lambs' nutrients intake (g/day) supplemented with different levels of zinc

Intake (g/day)	T1	T2	T3	SED	P-value
DM (Kg/day)	1.07	1.07	0.93	0.138	0.521
OM (Kg/day)	1.01	1.02	0.88	0.130	0.532
Protein (g/day)	141	141	123	18.15	0.498
CF (g/day)	169	169	147	21.74	0.547
ADF (g/day)	440	450	392	58.70	0.571
NDF (g/day)	470	472	410	60.60	0.487

T1= 0 mg Zn/day (treatment one), T2= 36 mg Zn/day (treatment two), T3= 72 mg Zn/day (treatment three). Means with different superscript letters differ ($P<0.05$). SED= standard error of deviation.

Table (4): Lambs' nutrients output (g/day) supplemented with different levels of zinc

Fecal output	T1	T2	T3	SED	P-value
DM (Kg/day)	0.38	0.32	0.28	0.056	0.235
OM (Kg/day)	0.29	0.25	0.237	0.049	0.494
Protein(g/day)	77.4	59.9	58.1	12.73	0.267
CF(g/day)	66.3b	62.4b	43.8a	8.418	0.031
ADF(g/day)	169b	134a	129a	32.96	0.425
NDF(g/day)	199b	132a	110a	29.02	0.014

T1= 0 mg Zn/day (treatment one), T2= 36 mg Zn/day (treatment two), T3= 72 mg Zn/day (treatment three). Means with different superscript letters differ ($P<0.05$). SED= standard error of deviation.

Table (5): Lambs' nutrients digestibility coefficient supplemented with different levels of zinc

Digestibility (g/g)	T1	T2	T3	SED	P-value
DM	0.64	0.71	0.69	0.038	0.091
OM	0.70	0.76	0.72	0.041	0.357
Protein	0.54	0.60	0.60	0.050	0.406
CF	0.61	0.66	0.68	0.058	0.163
ADF	0.54a	0.67b	0.65b	0.048	0.020
NDF	0.56a	0.73b	0.72b	0.048	0.004

T1= 0 mg Zn/day (treatment one), T2= 36 mg Zn/day (treatment two), T3= 72 mg Zn/day (treatment three). Means with different superscript letters differ ($P<0.05$). SED= standard error of deviation.

CONCLUSIONS

Zinc supplementation in current experiment was insufficient to increase blood serum concentration of Zn to the levels of previous published experiments that conducted out of hot season. This means that the requirements of Zn might be higher during hot season comparing to other seasons and need further work. Zn supplementation also increased serum content of ALP which means that Zn was effective in reducing the negative impact of heat stress. Zinc supplementation has improved the digestibility by reducing the effect of heat stress.

ACKNOWLEDGMENT

The author would like to appreciate and thank the Animal Production Department, College of Agricultural Engineering Sciences, University of Duhok for their help in providing the required equipment and experiment hall to conduct this experiment.

CONFLICT OF INTEREST

The authors stated that there are no conflicts of interest with the publication of this work.

تأثير إضافة الزنك على هضم العناصر الغذائية ومستوى المعادن في الدم خلال الموسم الحار للحملان المحلية

اراز قاسم سليمان [1]

قسم الإنتاج الحيواني / كلية علوم الهندسة الزراعية / جامعة دهوك / دهوك / العراق [1]

الخلاصة

أجريت هذه التجربة لتقييم تأثير إضافة عنصر الزنك على هضم العناصر الغذائية ومستوى العناصر المعدنية في الدم خلال الموسم الحار في الحملان العواسية المحلية. في هذه التجربة تم إيواء سبعة وعشرين حملاً محليًا بوزن حي 25.92 كغم (الانحراف المعياري= ± 4.29) وعمر 8 أشهر (الانحراف المعياري 1.1 = ±) في أقفاص فردية (1.50 × 1.30 م) داخل حضيرة مغلقة بشكل عشوائي لواحدة من ثلاث معاملات تجريبية. تمت إضافة الزنك إلى ماء الشرب بثلاثة مستويات مختلفة، 0 مل (0 ملغم / يوم)، 3 مل (36 ملغم زنك / يوم) و6 مل (72 ملغم / يوم) كمعاملات تجريبية الأولى (T1)، الثانية (T2) والثالثة (T3) على التوالي .تم تأقلم الحملان للمعاملات التجريبية لمدة 3 أسابيع، تلتها 5 أيام جمع عينات العلف والبراز في الأسبوع الرابع لإجراء المزيد من التحليلات المختبرية لتحديد معامل هضم العناصر الغذائية .في اليوم الثالث من أسبوع جمع العينات، تم سحب عينات الدم عن طريق الوريد الوداجي في الساعة 11 صباحًا لتقدير العناصر المعدنية والكيمياء الحيوية في الدم. أظهرت النتائج أن إضافة الزنك تزيد من تركيز الزنك في الدم .بالإضافة إلى ذلك، أدت مكملات الزنك إلى انخفاض كل من الالبومين وكلوكوز الدم في المعاملة التجريبية T3، في حين زاد تركيز ALP في الدم في المعاملة التجريبية T3. كانت إضافة الزنك فعالة في تحسين معامل هضم العناصر الغذائية وزيادة هضم كل من NDF وADF ومالت إلى زيادة هضم المادة الجافة. تشير النتائج المذكورة أعلاه إلى أن إضافة الزنك قد يكون لها دور في تقليل التأثير السلبي للإجهاد الحراري وتحسين معامل الهضم.

الكلمات المفتاحية: الإجهاد الحراري، الزنك، العناصر المعدنية، كيمياء الدم، معامل الهضم.

REFERENCES

Abbi, A. (2020). *Factors Affecting the Response of Pregnant and Lactating Ewes to Vitamin E Supplementation*. (Doctoral dissertation, Harper Adams University). https://hau.repository.guildhe.ac.uk/id/eprint/17654/

Abbi, A. Q. S., Zebari, H. M. H., Barwary, M. S. Q., & Hidayet, H. M. (2022). Effect of zinc addition to drinking water on performance and behavior of growing lambs during hot climate. *Iraqi Journal of Agricultural Sciences*, *53*(5), 1145–1153.https://www.iasj.net/iasj/download/febd9a78b6041bb2

Abdul-Rahman, S. Y., Rahawi, G. A., & Abdul-Majeed, A. F. (2022). Physiological aspects of phytochemicals as antioxidants on poultry:(Article Review). *Mesopotamia Journal of Agriculture*, *50*(3), 81–96. https://doi.org/10.33899/MAGRJ.2022.135167.1193

Abeni, F., Calamari, L., & Stefanini, L. (2007). Metabolic conditions of lactating Friesian cows during the hot season in the Po valley. 1. Blood indicators of heat stress. *International Journal of Biometeorology*, *52*(2), 87–96. https://doi.org/10.1007/s00484-007-0098-3

AFRC. (1993). *Energy and Protein Requirements of Ruminants.* An Advisory Manual Prepared by the AFRC Technical Committee on Responses to Nutrients.

Ahmed, S. J., & Abdul-Rahman, S. Y. (2023). Effects of castration and sex hormones on antioxidant status and some biochemical parameters of male rabbits exposed to oxidative stress. *Mesopotamia Journal of Agriculture*, *51*(1), 92–114. https://doi.org/10.33899/MAGRJ.2023.138538.1220

Aliarabi, H., Fadayifar, A., Tabatabaei, M. M., Zamani, P., Bahari, A., Farahavar, A., & Dezfoulian, A. H. (2015). Effect of Zinc Source on Hematological, Metabolic Parameters and Mineral Balance in Lambs. *Biological Trace Element Research*, *168*(1), 82–90. https://doi.org/10.1007/s12011-015-0345-0

Alimohamady, R., Aliarabi, H., Bahari, A., & Dezfoulian, A. H. (2013). Influence of different amounts and sources of selenium supplementation on performance, some blood parameters, and nutrient digestibility in lambs. *Biological Trace Element Research*, 154(1), 45–54. https://doi.org/10.1007/s12011-013-9698-4

Alimohamady, R., Aliarabi, H., Bruckmaier, R. M., & Christensen, R. G. (2019). Effect of Different Sources of Supplemental Zinc on Performance, Nutrient Digestibility, and Antioxidant Enzyme Activities in Lambs. *Biological Trace Element Research*, 189(1), 75–84. https://doi.org/10.1007/s12011-018-1448-1

Anonymous. (1981). National Research Council NRC. *Effect of Environment on Nutrient Requirements of Domestic Animals*. National Academies Press. Washington, DC. https://nap.nationalacademies.org/download/4963

Anonymous. (2005). National Research Council NRC. *Mineral Tolerance of Animals* (Second Revised Edition). Board on Agriculture, Division on Earth, & Life Studies.National Academies Press. Washington, DC. https://doi.org/10.17226/11309

Anonymous. (2007). National Research Council NRC. *Committee on Nutrient Requirements of Small Ruminants*. Board on Agriculture, Division on Earth, & Life Studies. National Academies Press. Washington, DC. https://doi.org/10.17226/11654

AOAC. (2016). *Official Methods of Analysis of AOAC International.* (20th ed.).

Attia, N. E. S. (2016). Physiological, hematological and biochemical alterations in heat stressed goats. *Benha Veterinary Medical Journal*, 31(2), 56–62. http://www.bvmj.bu.edu.eg

Bao, B., Ahmad, A., Azmi, A., Li, Y., Prasad, A., & Sarkar, F. H. (2014). The Biological Significance of Zinc in Inflammation and Aging. *Inflammation, Advancing Age and Nutrition: Research and Clinical Interventions*, 15–27. https://doi.org/10.1016/B978-0-12-397803-5.00002-2

Baumgard, L. H., & Rhoads, R. P. (2013). Effects of heat stress on postabsorptive metabolism and energetics. *Annual Review of Animal Biosciences*, 1(1), 311–337. https://doi.org/10.1146/annurev-animal-031412-103644

Beede, D. K., & Collier, R. J. (1986). Potential nutritional strategies for intensively managed cattle during thermal stress. *Journal of Animal Science*, *62*(2), 543–554. https://doi.org/10.2527/jas1986.622543x

Bernabucci, U., Bani, P., Ronchi, B., Lacetera, N., & Nardone, A. (1999). Influence of short- and long-term exposure to a hot environment on rumen passage rate and diet digestibility by friesian heifers. *Journal of Dairy Science*, *82*(5), 967–973. https://doi.org/10.3168/jds.S0022-0302(99)75316-6

Bernabucci, U., Lacetera, N., Danieli, P. P., Bani, P., Nardone, A., & Ronchi, B. (2009). Influence of different periods of exposure to hot environment on rumen function and diet digestibility in sheep. *International Journal of Biometeorology*, *53*(5), 387–395. https://doi.org/10.1007/s00484-009-0223-6

Block, E., Kilmer, L. H., & Muller, L. D. (1981). Acid insoluble ash as a marker of digestibility for sheep fed corn plants or hay and for lactating dairy cattle fed hay ad libitum. *Journal of Animal Science*, 52(5), 1164–1169. https://academic.oup.com/jas/article-abstract/52/5/1164/4658388

Chavan, S. J., Varadan, D., Ravishankar, C., Vazhoor, B., Sebastian, R., Chulliparambil, S., & Prakash, P. (2021). The effect of inorganic and organic zinc supplementation on growth performance, mineral profile and gene expression pattern of GLUT1 in malabari kids. *Biological Trace Element Research*, *199*(2), 568–577. https://doi.org/10.1007/s12011-020-02167-y

Cho, Y.E., Lomeda, R.A.R., Ryu, S.H., Sohn, H.Y., Shin, H.I., Beattie, J. H., & Kwun, I.S. (2007). Zinc deficiency negatively affects alkaline phosphatase and the concentration of Ca, Mg and P in rats. *Nutrition Research and Practice*, 1(2), 113-119. https://synapse.koreamed.org/articles/1050730

Fuquay, J. W. (1981). Heat stress as it affects animal production. *journal of animal science*. *52*(1), 164–173. https://academic.oup.com/jas/article-abstract/52/1/164/4658243

Garg, A. K., Mudgal, V., & Dass, R. S. (2008). Effect of organic zinc supplementation on growth, nutrient utilization and mineral profile in lambs. *Animal Feed Science and Technology*, 144(1–2), 82–96. https://doi.org/10.1016/j.anifeedsci.2007.10.003

Ghassemi Nejad, J., Lohakare, J. D., West, J. W., & Sung, K. I. (2014). Effects of water restriction after feeding during heat stress on nutrient digestibility, nitrogen balance, blood profile and characteristics in corriedale ewes. *Animal Feed Science and Technology*, *193*, (1–8). https://doi.org/10.1016/j.anifeedsci.2014.03.011

Hassan, E. H., Farghaly, M.M., & Solouma, G. M. (2016). Effect of zinc supplementation from inorganic and organic sources on nutrient digestibility, some blood metabolites and growth performance of growing buffalo calves.

Egyptian Journal of Nutrition and Feeds, *19*(1), 37-46. https://doi.org/10.21608/ejnf.2016.74863

Hosseini-Vardanjani, S. F., Rezaei, J., Karimi-Dehkordi, S., & Rouzbehan, Y. (2020). Effect of feeding nano-ZnO on performance, rumen fermentation, leukocytes, antioxidant capacity, blood serum enzymes and minerals of ewes. *Small Ruminant Research*, 191(1) 1-8. https://doi.org/10.1016/j.smallrumres.2020.106170

Kadzere, C. T., Murphy, M. R., Silanikove, N., & Maltz, E. (2002). Heat stress in lactating dairy cows: a review. *Livestock Production Science*, 77(1), 59-91. www.elsevier.com/locate/livprodsci

Kaki, S., Moeini, M. M., & Hozhabri, F. (2020). Effects of mixed crushed caraway (Carum carvi) with chromium-methionine or zinc-methionine supplementations on serum components and physiological responses of lambs subjected to transportation stress. *Small Ruminant Research*, *183*, 106040 (1-27). https://doi.org/10.1016/j.smallrumres.2019.106040

Kumar, N., Verma, R. P., Singh, L. P., Varshney, V. P., & Dass, R. S. (2006). Effect of different levels and sources of zinc supplementation on quantitative and qualitative semen attributes and serum testosterone level in crossbred cattle (Bos indicus × Bos taurus) bulls. *Reproduction Nutrition Development*, 46(6), 663–675. https://doi.org/10.1051/rnd:2006041

Liu, H. Y., Sun, M. H., Yang, G. Q., Jia, C. L., Zhang, M., Zhu, Y. J., & Zhang, Y. (2015). Influence of different dietary zinc levels on cashmere growth, plasma testosterone level and zinc status in male Liaoning Cashmere goats. *Journal of Animal Physiology and Animal Nutrition*, *99*(5), 880–886. https://doi.org/10.1111/jpn.12292

Mallaki, M., Norouzian, M. A., & Khadem, A. A. (2015). Effect of organic zinc supplementation on growth, nutrient utilization, and plasma zinc status in lambs. *Turkish Journal of Veterinary and Animal Sciences*, 39(1), 75–80. https://doi.org/10.3906/vet-1405-79

Mandal, G. P., Dass, R. S., Garg, A. K., Varshney, V. P., & Mondal, A. B. (2008). Effect of zinc supplementation from inorganic and organic sources on growth and blood biochemical profile in crossbred calves. *Journal of Animal and Feed Sciences*, 17(2), 147-156. DOI: https://doi.org/10.22358/jafs/66478/2008

Mandal, G. P., Dass, R. S., Isore, D. P., Garg, A. K., & Ram, G. C. (2007). Effect of zinc supplementation from two sources on growth, nutrient utilization and immune response in male crossbred cattle (Bos indicus×Bos taurus) bulls. *Animal Feed Science and Technology*, *138*(1), 1–12. https://doi.org/10.1016/j.anifeedsci.2006.09.014

Mathers, J. C., Baberf, R. P., & Archibald, R. F. (1989). Intake, digestion and gastro-intestinal mean retention time in Asiatic buffaloes and Ayrshire cattle given two contrasting diets and housed at 20° and 33 °C. *The Journal of Agricultural Science*, 113(2),211-222. https://doi.org/10.1017/S0021859600086792

McDowell, L., Wilkinson, N., Madison, R., & Felix, T. (2007). Vitamins and minerals functioning as antioxidants with supplementation considerations. *Florida Ruminant Nutrition Symposium*, 352(3), 1–17. https://2u.pw/PyUUwr9

Miaron, J. O. O., Christophersonl, R. L., & Christopherson, J. O. (1992). Effect of prolonged thermal exposure on heat production, reticular motility, rumen-fluid and-partiqrlate passa ge-r ate constants, and apparent digestibility in steers. *Canadian Journal of Animal Science*, *7*(4), 809–819. https://doi.org/10.4141/cjas92-093

Minervino, A. H. H., López-Alonso, M., Barrêto Júnior, R. A., Rodrigues, F. A. M. L., Araújo, C. A. S. C., Sousa, R. S., Mori, C. S., Miranda, M., Oliveira, F. L. C., Antonelli, A. C., & Ortolani, E. L. (2018). Dietary zinc supplementation to prevent chronic copper poisoning in sheep. *Animals*, *8*(12) 227. https://doi.org/10.3390/ani8120227

Nasrollahi, S. M., Zali, A., Ghorbani, G. R., Khani, M., Maktabi, H., & Beauchemin, K. A. (2019). Effects of increasing diet fermentability on intake, digestion, rumen fermentation, blood metabolites and milk production of heat-stressed dairy cows. *Animal*, 13(11), 2527–2535. https://doi.org/10.1017/S1751731119001113

Nosir, W. (2023). Climate change: consequences on Iraq's environment. *Mesopotamia Journal of Agriculture*, *51*(2), 131–146. https://doi.org/10.33899/MAGRJ.2023.140391.1243

Omar, D. E., & Barwary, M. S. Q. (2022). Physiological Response of Lamb to Zinc Supplementation during Heat Stress Season. *The Journal of Duhok University*, *25*(1), 141–150. https://doi.org/10.26682/ajuod.2022.25.1.14

Rahawi, G. A. M., Abdul-Majeed, A. F., & Abdul-Rahman, S. Y. (2022). The role of antioxidant vitamins on physiological performance of poultry (article review). *Mesopotamia Journal of Agriculture*, 50(1), 65–77. https://doi.org/10.33899/MAGRJ.2022.133151.1167

Rhoads, M. L., Rhoads, R. P., VanBaale, M. J., Collier, R. J., Sanders, S. R., Weber, W. J., Crooker, B. A., & Baumgard, L. H. (2009). Effects of heat stress and plane of nutrition on lactating Holstein cows: I. Production, metabolism, and aspects of circulating somatotropin. *Journal of Dairy Science*, 92(5), 1986–1997. https://doi.org/10.3168/jds.2008-1641

Saker, K. E., Fike, J. H., Veit, H., & Ward, D. L. (2004). Brown seaweed- (Tasco^{TM}) treated conserved forage enhances antioxidant status and immune function in heat-stressed wether lambs. *Journal of Animal Physiology and Animal Nutrition*, 88(3–4), 122–130. https://doi.org/10.1111/j.1439-0396.2003.00468.x

Silanikove, N. (1992). Effects of water scarcity and hot environment on appetite and digestion in ruminants: a review. *Livestock Production Science*, 30(3), 175-194. https://doi.org/10.1016/S0301-6226(06)80009-6

Song, C., & Shen, X. (2020). Effects of Environmental Zinc Deficiency on Antioxidant System Function in Wumeng Semi-fine Wool Sheep. *Biological Trace Element Research*, 195(1), 110–116. https://doi.org/10.1007/s12011-019-01840-1

Spears, J. W., & Weiss, W. P. (2008). Role of antioxidants and trace elements in health and immunity of transition dairy cows. *Veterinary Journal*, 176(1), 70–76. http://dx.doi.org/10.1016/j.tvjl.2007.12.015

Sunil Kumar, V. B., Ajeet, K., & Meena, K. (2011). Effect of Heat Stress in Tropical Livestock and Different Strategies for Its Amelioration. *Journal of Stress Physiology & Biochemistry*, *7*(1), 45–54. https://2u.pw/p4qI1XQ

Suttle NF. (2010). *Mineral Nutrition of Livestock (fourth edition)*. CAB International, Wallingford,UK. Mineral Nutrition of Livestock.pdf

Sweeney, R. A. (1989). Generic combustion method for determination of crude protein in feeds: Collaborative study. *Journal-Association of Official Analytical Chemists*, *72*(5), 770–774. https://doi.org/10.1093/jaoac/72.5.770

Van Keulen, J., & Young, B. A. (1977). Evaluation of acid-insoluble ash as a natural marker in ruminant digestibility studies. *Journal of Animal Science*, *44*(2), 282–287. https://doi.org/10.2527/jas1977.442282x

VanValin, K. R., Genther-Schroeder, O. N., Carmichael, R. N., Blank, C. P., Deters, E. L., Hartman, S. J., Niedermayer, E. K., & Hansen, S. L. (2020). Trace mineral metabolism and nutrient digestibility in lambs supplemented with zinc sulfate during an adrenocorticotropic hormone challenge. *Livestock Science*, 241, 104197 (1-7). https://doi.org/10.1016/j.livsci.2020.104197

Wheelock, J. B., Rhoads, R. P., VanBaale, M. J., Sanders, S. R., & Baumgard, L. H. (2010). Effects of heat stress on energetic metabolism in lactating Holstein cows. *Journal of Dairy Science*, *93*(2), 644–655. https://doi.org/10.3168/jds.2009-2295

Yadav, B., Singh, G., Wankar, A., Dutta, N., Chaturvedi, V. B., & Verma, M. R. (2016). Effect of simulated heat stress on digestibility, methane emission and metabolic adaptability in crossbred cattle. *Asian-Australasian Journal of Animal Sciences*, *29*(11), 1585–1592. https://doi.org/10.5713/ajas.15.0693