https://doi.org/10.26682/ajuod.2022.25.1.14
Journal of University of Duhok.,Vol. 25, No.1 (Agri. and Vet. Sciences), Pp 141-150, 2022

PHYSIOLOGICAL RESPONSE OF LAMB TO ZINC SUPPLEMENTATION DURING HEAT STRESS SEASON

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(Received: April 27, 2022; Accepted for Publication: June 12, 2022)

ABSTRACT

The aim of this research was to evaluate the role of zinc supplementation on the reduction of heat stress in local lambs during hot season. In the current experiment, twenty-seven lambs were housed indoor in individual pens (1.50 × 1.30m). Zinc was added to the fresh drinking water in three different levels namely T_1 (0 mg Zn/day), T_2 (36 mg Zn/day) and T_3 (72 mg Zn/day). Serum zinc (zinc; µmol/L), Alkaline phosphate (ALP; U/I), Alanine amino transferase (ALT; U/I), Aspartate amino transferase (AST; U/I), Creatine kinase (CK; U/I), Blood glucose level (mg/dl), Triiodothyronine hormone (T3; ng/ml), Thyroxin hormone (T4; ng/ml) were measured once per week. Zinc supplementation results show significant decrease in Aspartate amino transferase (AST) in (week 4), and Creatine kinase (CK) at (week 6) in T1group. Also zinc supplementation decreased triiodothyronine hormone (T3), and thyroxin hormone (T4) in (week 4). Whereas serum zinc level and alkaline phosphate (ALP) increased, also blood glucose level increased significantly in T2 (week 5) when the lambs were supplemented with 36 mg / zinc/ day. However, no effect of zinc supplementation was recorded on lamb serum ALT. Zinc supplementation was effective to increase blood plasma zinc concentration, ALP and kept the level of T4 from reduction during heat stress. However, zinc supplementation failed to keep the level of T3 from reduction during heat stress. This could be due to the type of zinc that was supplemented in the current experiment was inorganic which is less bioavailable than organic zinc.

***KEYWORDS*:** Zinc; heat stress; serum biochemical; serum plasma; serum enzymes.

INTRODUCTION

Heat stress plays a significant role in enhancing oxidative stress either via reduction antioxidant defenses or excessive production of reactive oxygen species (ROS) (Trout *et al.,* 1998; Bernabucci *et al.,* 2002; Saker *et al*., 2004; Di Trana *et al.,* 2006). Extreme production of ROS makes the antioxidant defenses to be swept up which results in oxidative damage of biological molecules including proteins, lipids, and DNA (Machlin and Bendich, 1987; Halliwell and Gutteridge, 1990), hampering regular metabolism and physiology (Trevisan *et al.,* 2001).

Body employs antioxidants to reduce the free radicals of reactive oxygen species (ROS) (Sunil Kumar *et al.,* 2011). Antioxidant nutrient supplementation especially vitamins C, A and E, Zn, and chromium can be used to decrease the negative effects of environmental stress (Sunil Kumar *et al.,* 2011). The metalloenzymes which contain glutathione peroxidase (Se), superoxide dismutase (Zn and Cu) and catalase (Fe), are regarded to be intracellular antioxidant enzymes. These enzymes transform the hydrogen peroxide (H_2O_2) to alcohol and water and preserve intracellular constituents from oxidative damage (McDowell *et al.,* 2007; Spears and Weiss, 2008). The antioxidant enzymes activity and the blood substance of mineral elements are nearly related (Song and Shen, 2020). As an antioxidant, Zn has an important role in the body's antioxidant system, averting oxidation of cell membrane and reduce the super anions and cations to be formed (Song and Shen, 2020). Therefore, zinc supplementation might reduce oxidative stress of lambs that are in the process of growing (Liu *et al.,* 2014).

Tolerance to high Zn intake is lower in ruminants than the other animals (NRC, 2007a). The maximum tolerance diet content of Zn for sheep is 300 mg/kg DM (NRC, 2005). The

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National Research Council ((NRC, 2007) recommends that animal diets should be supported with zinc. According to NRC (2007), the recommended level of Zn supplementation is about 40 mg/day for lambs with 30 to 50 kg BW and an ADG of 250 g/day. However, Alimohamady *et al.,* (2019a) suggested that the requirements of Zn may be higher than NRC (2007) recommendations (75 mg Zinc/day).

In Iraq, Naji (2017) found that 60 from 75 sheep had clinical sings of Zn deficiency (hypoZincemia) in Basra province. Naji (2017) also documented a reduction in glutathione because of zinc deficiency. This means that the sheep in Iraq might have zinc deficiency. Depending on this study and its outcome, the possibility of local sheep suffering from zinc deficiency could be a fact. According to our knowledge, it appeared that there might be a possibility of having no researches about this field in the Kurdistan Region. And if there is such research, it is not definite if these researches have depended and/or followed the NRC (2007) recommendations. This could need further investigation particularly during heat stress season. Therefore, the aim of current study is to evaluate the role of zinc supplementation on the reduction of heat stress in lamb during hot season.

MATERIALS AND METHODS

The experiment was undertaken between July 1st – September 1st, 2020 at Animals Farm Project, Department of Animal Production, College of Agriculture Engineering Sciences, University of Duhok. The Department of Animal Production Research Ethics Committee approved the research protocol.

Experimental animals

A total of twenty-seven local male lambs aged (±SEM) 8 ± 1.1 months with a 25.9 kg ± SD= 4.22 live body weight were used at the farm of the Department of Animal Production. The lambs were housed indoor in individual pens (1.50 x 1.30m) in randomized blocks on sawdust. For adaptation, the lambs were moved into the study designated location one week before data collection. During the adaptation period, all lambs were drenched against the internal parasite and repeated 21 days later using Levazide forte by drenching, and external parasite using Cypervet 100 EC (cypermethrin, manufactured in Astral Industrial Complex Co.Ltd.Riyadh-Kingdom of Saudi Arabia.) at the start of the experiment by dipping. Before the experiment, C&D antitoxin (Polivac cl), 100ml, Manufactured By Vetal, Adiyaman-Turkey) was used to vaccinate the lambs against enterotoxemia. The lambs were fed with concentrate (400g) and hay twice a day, then the feeding meal quantity was increased gradually by (10%) until it reached ad-libitum feeding from the start date of experiment. After the adaptation period, two kg of a full mixed portion (TMR; see Table 1) was offered on everyday basis at around 09:00 a.m. and 05:00 p.m. for ad libitum availability using individual feed plastic buckets. Potable water was offered ad libitum daily using suitable plastic buckets.

Table (1): Dietary composition of the concentrate mixture diet.

Ingredients	g/kg (%)
Barley	550 (55%)
Corn	100 (10%)
Wheat barn	150 (15%)
Soybean meal	170 (17%)
Calcium Carbonate (CaCo3)	20.0 (2%)
Di Calcium Phosphate	4.00 (0.4%)
Salt	5.00 (0.5%)
Multi Vitamin (ADE)	1.00 (0.1%)
Total	1000 (100%)

Experimental design:

The (ACTIVE BIOTIN ZINC), (Zinc sulphate, monohydrate (12 mg/ml)) was added to the potable water in three different levels namely T1 (0 mg/day), T2 (36 mg/day) and T3 (72 mg/day).

Data from T1, T2 and T3 lambs were collected during the study period. Blood samples were collected weekly. The collected blood (7 ml) emptied into anticoagulant free test gel tube for 24 hours and stored in refrigerator, then centrifuged for 15 minutes at (3000 rpm), and the obtained serum stored in plastic Eppendorf tube at -20 C° for analyzing triiodothyronine (T_3), thyroxin (T_4), serum zinc concentration, Alkaline phosphate (ALP; U/I), Alanine amino transferase (ALT; U/I), Aspartate amino transferase (AST; U/I), Creatine kinase (CK; U/I), Blood glucose level (mg/dl), all the above mentioned parameters were determined by using FUJI DRI-CHEM SLIDE for auto biochemistry analyzer from Fujifilm DRI-CHEM NX500i, China. Blood serum Triiodothyronine hormone (T3; ng/ml) and Thyroxin hormone (T4; ng/ml) were also determined by automated method of biochemistry analyzer mini VIDAS device. Microsoft Excel tool was used in the summarization of all parameters' data into one value per week.

Statistical analyses

ANOVA factorial one way were used for analyzing the data by using the Genstat statistical software package (Genstat V 20th edition, VSN International Ltd, U.K.). The datasets were analyzed to compare among the experimental groups for the Alkaline phosphate (ALP; U/I), Alanine amino transferase (ALT; U/I), Aspartate amino transferase (AST; U/I), Creatine kinase (CK; U/I), Blood glucose level (mg/dl), Triiodothyronine hormone (T3; ng/ml), Thyroxin hormone (T4; ng/ml). Repeated measure ANOVA were used to compare between treated groups during different time (weeks) and the treatment x time interaction. Tukey test was applied to compare various groups for all parameters. Differences were reported as significant at P <0.05, and trends were reported when P was between <0.1 and >0.05. The time frame of week (0) has set as a covariate for blood serum zinc metabolites.

RESULTS AND DISCUSSTIONS

Biochemical parameters: There was a substantial impact of time (P<0.001) on lamb blood serum zinc concentration during hot climate season (Table 2). There was an increase in serum zinc level during experiment progress, however serum zinc level increased in T3 at week 6 in the comparison to other treatment groups. There was an effect of zinc supplementation on the level of serum zinc, Serum zinc was increased throughout the experiment period except first week (P=0.137) in T3 when the lambs were supplemented with 72 mg /Zinc/ day. The results of the present study agree with those published by Mallaki et al.(2015) showed that there was an increase in the Zn level in the serum in supplemented groups as (ZnP and ZnS) in juxtaposition to the control in lambs, this experiment is studied without heat stress conditions. The results of the present study disagree with the previously study carried out by Alimohamady et al.(2019) who observed that there were no significant differences of serum Zn concentrations between groups supplemented by organic Zn (Zn-methionine and Zn-proteinate supplementation) and inorganic Zn(Zn-sulfate) compared to control group in lambs under normal conditions. In agreement with the data published by Patel et al.(2018) who observed that serum Zn level was significantly higher in supplemented groups were fed with 80 ppm and 120 ppm Zn compared to control group in Karan Fries cows during heat stress.

concerning the level of ALT in serum is illustrated in (Table 4). There was a significant effect of time (P = 0.001) on serum ALT during hot climate. ALT level was decreased during the experimental progress; however, serum ALT was decreased in T3 group from week one to week six. There was no effect of zinc supplementation on serum ALT during hot climate. Consistent with the current study, Sun et al.(2019) showed that cows supplemented with hydroxy-selenomethionine (HMSeBA) as a source of organic Se had lower ALT activity than sodium selenite (SS; 0.3 mg of Se/kg) as asource of inorganic Se supplemented during hot climate. The organic zinc is more available than non-organic zinc (Garg et al., 2008; Mallaki et al. 2015). Probably the inorganic source of supplemented zinc in current study had no effect in ALT. Rathwa et al.(2017) and Nazifi et al.(2003) studies in sheep observed that the levels of blood ALT was significantly increased during heat stress conditions, this may be due to that these studies did not use any antioxidants supplementation. The concentrations of ALT in dairy cow blood were decreased ($p < 0.05$) in the Holstein cows under heat stress; however, in Jersey cows there was no change in ALT level in high heat environmental conditions (Joo et al.2021), these experiments also were conducted without the use of antioxidants. In contrast to the results of the current study, Alhidary et al.(2015) found that ALT concentration in serum were increased in sheep supplemented with Se and vitamin E during heat stress, this could be that Se and vitamin E better as antioxidants than Zn only under heat stress conditions. These changes in the blood concentrations of these enzymes could be caused by a reduction in thyroid hormone activity during high heat load (Collins and Weiner, 1968;Magdub et al.,1982). It has been revealed that there is a strong correlation between thyroid hormone activity and blood concentrations of the studied enzymes(Khan et al.2010).

The data related to the level of AST in serum is illustrated in (Table 5). A significant effect of time (P= 0.001) on serum AST was observed during the hot climate season. With the experimental development, the level of the AST trended to decrease in T3 in comparison to other groups. Zinc supplementation had a significant effect ($P < 0.05$) on serum AST. At week four, the level of AST was decreased (P = 0.030) and tended to be lower at week five (P = 0.090) in T1 compared to other studied groups. In agreement with the current study, AST activities decreased

(P< 0.01) in lambs (Mohri et al., 2011) and on sheep (Srikandakumar et al., 2003) in the groups that exposed to heat stress. Previous studies conducted on sheep by Rathwa et al.(2017) and Nazifi et al.(2003) reported higher activities of AST during heat stress conditions, Furthermore, Joo et al.(2021) reported that there was no difference in AST level in cows circulating blood in high temperature conditions, this could be these experiments were conducted without the use of antioxidants. Song and Shen (2020) confirmed that a high serum level of AST enzyme occur in disorders or conditions that involve hepatocyte or muscle damage. Aspartate aminotransferase is known to be a cytoplasmic and mitochondrial enzyme which catalyzes a reversible reaction involved in the deamination of aspartate to form oxaloacetate, which can enter the Krebs cycle (Song and Shen, 2020). Therefore, Zn supplementation due to increase in its serum concentration can determine the efficiency of AST enzyme activity in circulating blood of lamb (Song and Shen, 2020).

The data regarding to CK level in the present study see (Table 6). There was a significant effect of time (P<0.001) on serum CK during the hot climate. With the experimental progress the level of CK reduction was higher in T2 in comparison to other groups. Zinc supplementation had also significant effect (P < 0.05) on serum CK. At weeks six, the level of CK of T2 serum was decreased (P = 0.008) and tended to be lower at week zero (P = 0.058) in T1. In contrary with the current study, results reported by Sun et al.(2019) who observed that selenium has no effects on CK level (P > 0.05) on cows exposed to heat stress, the reason could be that zinc bioavailability is better as antioxidants than Se. In agreement with the current study, Joo et al. (2021) reported that heat stress resulted in decreased concentrations of serum enzyme CK in both the Holstein and Jersey cows. In contrast to the current study, previous study conducted by Alhidary et al.(2015) reported that CK had not effected by Se and vitamin E supplementation during heat stress in sheep. This could be due to that zinc bioavailability is better as antioxidants than Se. The current study disagrees with the finding of (Burk, 2007) who indicated that increases in CK activities in the blood of sheep and cattle were associated with a diet deficient in Se and vitamin E during normal environmental. This suggests that the concentration of serum CK of lamb supplemented with zinc in the current study was reduced, possibly caused by improved antioxidant status during the hot climate.

There was a significant effect of time (P<0.001) on lamb glucose during the hot climate as shown in (Table 7). There was fluctuation in blood glucose level during experimental progress. Zinc supplementation had a significant effect (P < 0.05) on lamb glucose. At week five in T2, the level of glucose was considerably increased (P = 0.005). The results reported by (Alkass, 2017) disagreed with the findings of this study, he showed that Meriz goats received high level of Se and vit. E had significantly lower level of glucose during hot climate, the reason could be the different type of antioxidants or different studied breed of animal. In contrast Sun et al.(2019) observed that Se source as antioxidant had no effects on serum glucose in dairy cows exposed to heat stress, the reason could be the breed or the Zn bioavailability is better than Se. Whereas, Srikandakumar et al.(2003) reported that heat stress increased serum glucose in Merino while found that glucose was decreased in Omani sheep during heat stress. Also, Okoruwa, (2014) reported higher concentration of blood glucose during hot climate in goats. On the other hand, it has been reported in previous studies conducted without the use of antioxidant and found that the level of glucose were significantly decreased during hot climate in goats by Attia, (2016), in sheep by (Rathwa et al., 2017; Nazifi et al., 2003), and in cows by Joo et al. (2021). Several studies demonstrated that heat stress reduced serum glucose (Abeni et al., 2007; Wheelock et al., 2010), as well as causing oxidative stress and reducing serum antioxidant activity (Bernabucci et al., 2002). Inconsistent with the current study, Attia(2016) found that glucose concentrations decreased markedly, along with high ambient temperature, and this may be due to greater activity of blood insulin (Baumgard and Rhoads, 2013; Rhoads et al., 2009) or could be due to reduced feed intake (Rhoads et al., 2009).

There was an effect of time (P < 0.003) on lamb T3 during hot climate season (Table 8). T3 was increased with experiment progress; however, triiodothyronine was slightly increased and its levels was lower in T3 treatment compared to the other treatments. There was an effect (P < 0.01) of zinc supplementation on serum T3. SerumT3 was lower at week three (P = 0.024), four (P =0.024); and trended to be lower at week two (P =0.059) and week five (P =0.074) when the lambs were supplemented with 72 mg Zinc/ day compared to other groups. This result was in

contrast with the study of Kb et al.(2020) who showed increase ($P < 0.05$) in serum T3 concentration in Mandya sheep supplemented with Selenium and Zinc (150 Ppm/animal/day) in heat stress, this may be due to that these study used different levels of zinc supplementation in comparing to the current study. In contrast with the current study, Nazifi et al.(2003) sheep, and Chen et al.(2018) on cows, observed a reduction in level of triiodothyronine during heat stress without the use of antioxidants. Discrepancy to the current study, Shakirullah et al.(2017) on sheep, Tejaswi et al.(2020) on cows showed that the concentration of T3 was significantly increased for animals exposed to heat stress. However heat stress did not result in significant changes in the thyroid hormones concentrations for Naimey sheep reported by Al-Haidary(2004), these experiments were conducted without antioxidants.

Furthermore, there was a significant effect of time ($P < 0.001$) on lamb T4 during the hot climate (Table 9). T4 level was increased during the experimental development, however the level of T4 recorded was lower in T3 treatment compared to the other treatment groups. Zinc supplementation had a significant effect ($P < 0.05$) on serum T4. At week four, the level of T4 was decreased ($P = 0.024$) in T3 compared to other groups. This result disagrees with that reported by Sejian et al.(2014) who found that the serum concentration of total thyroxin hormone was reduced by Vit E and Se supplementation in Malpura ewes, the reason could be that zinc bioavailability is better as antioxidants than Vit E and Se. However the current study agree with data reported by Kb et al.(2020) in sheep who found an increase in T4 level in groups supplemented with(Vit C, Vit E, Se, and Zn) in the form of powder under heat stress condition. And with Shakirullah et al.(2017) in sheep who showed significant ($P < 0.05$) increase in T4 level in sheep groups supplemented with Vit E and Selenium under heat stress condition. Exposure to heat stress challenge did not result in significant changes in the thyroid hormones concentrations for Naimey sheep (Al-Haidary,2004), this experiment disagree with current study because it was conducted without use of antioxidants. In contrast with the current results, a reduction in the level of thyroxine concentration was recorded in sheep (Rathwa et al., 2017; Nazifi et al., 2003) and dairy cows (Chen et al., 2018) in animals that exposed to heat stress without the using antioxidants. Furthermore in contrast to the present study, earlier study by Alimohamady et al.(2019) found that the thyroxine level in groups supplemented with organic zinc (Zn- methionine and Zn-glycine) was significantly higher in comparison to those supplemented with inorganic zinc (Zn-proteinate and Zn-sulfate) during normal environmental conditions. The effect of Serum zinc on thyroid hormones are complex including both synthesis and mode of action (Ertek et al., 2010). However, the results of the present study did not support the concept that organic zinc may be metabolized differently from inorganic Zn and, thus, may alter some metabolic processes differently (Spears, 1996).

Conclusion: Zinc supplementation was effective to increase blood serum zinc concentration, ALP and kept the level of T4 from decline during heat stress. However, zinc supplementation failed to keep the level of T3 from reduction during heat stress. This could be due to the type of zinc that was supplemented in the current experiment was inorganic which is less bioavailable than organic zinc.

Table (2): The effect of zinc (Zn) supplementation on lamb blood serum zinc concentration (µmol/L) during hot climate season.

Weeks	T1	T2	T3	SED	P-value
W1	7.26	6.59	5.81	0.537	0.137
W2	7.44b	8.21a	8.47a	0.1434	<0.001
W3	6.28c	6.64b	6.96a	0.1271	<0.001
W4	7.56	7.47b	8.20a	0.2796	0.013
W5	7.29b	9.09a	8.81a	0.831	0.037
W6	8.12b	11.69a	12.44a	0.715	<0.001

T1= treatment one ((no zinc supplementation) (0 mg Zn/day)), T2= treatment two (36 mg Zn/day), T3= treatment three (72 mg Zn/day), Means with different superscript letters differ (P<0.05). SED=standard error of deviation, Repeated measurements analysis: SED values: T= 0.240, Time= 0.196, T×Time=0.374; P-values: T= P<0.001, Time= P<0.001, Time×T= P<0.001.

Table (3): The effect of zinc (Zn) supplementation on lamb ALP (U/I) during hot climate season

weeks	T1	T2	T3	SED	P-value
W0	78.0	93.1	84.7	9.880	0.326
W1	102a	139b	123ab	13.97	0.050
W2	124a	188b	122a	24.34	0.020
W3	184	257	188	32.10	0.058
W4	180a	319b	207a	38.60	0.004
W5	207a	309b	177a	37.40	0.005
W6	218	199	118	48.40	0.117

T1= treatment one ((no Zinc supplementation) (0 mg Zn/day)), T2= treatment one (36 mg Zn/day), T3= treatment three (72 mg Zn/day), Means with different superscript letters differ (P<0.05). SED=standard error of deviation, Repeated measures analysis: SED values: T= 19.58, Time= 15.25, T×Time=31.33 P-values: T= 0.003, Time= 0.001, Time×T=0.018.

Table (4) :The effect of zinc (Zn) supplementation on lamb ALT (U/I) during hot climate season

Weeks	T1	T2	T3	SED	P-value
W0	19.2	18.7	22.0	2.249	0.302
W1	16.7	16.2	18.0	1.935	0.639
W2	13.4	13.5	15.3	1.461	0.375
W3	17.4	14.5	15.4	1.965	0.325
W4	17.4	17.5	18.6	2.285	0.861
W5	17.6	17.9	18.7	2.390	0.892
W6	16.6	14.1	15.5	1.687	0.375

T1= treatment one ((no Zinc supplementation) (0 mg Zn/day)), T2= treatment two (36 mg Zn/day), T3= treatment three (72 mg Zn/day), Means with different superscript letters differ (P<0.05). SED=standard error of deviation, Repeated measurements analysis: SED values: T= 1.630, Time= 0.851, T×Time=2.125 P-values: T= 0.870, Time= 0.001, Time×T=0.580.

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Table (5): The effect of zinc (Zn) supplementation on lamb AST (U/I) during hot climate season

weeks	T1	T2	T3	SED	P-value
W0	100	104	103	11.31	0.935
W1	78.0	79.0	85.6	8.800	0.647
W2	71.2	57.0	65.4	8.210	0.240
W3	77.9	81.0	73.7	6.850	0.576
W4	92.7a	123b	91.9a	12.12	0.030
W5	91.5	119	116	12.84	0.090
W6	92.4	106	104	18.73	0.731

T1= treatment one ((no Zinc supplementation) (0 mg Zn/day)), T2= treatment two (36 mg Zn/day), T3= treatment three (72 mg Zn/day), Means with different superscript letters differ (P<0.05). SED=standard error of deviation, Repeated measurements analysis: SED values: T= 6.70, Time= 6.19, T×Time= 11.97 P-values: T= 0.641, Time= 0.001, Time×T=0.281.

Table (6): The effect of zinc (Zn) supplementation on lamb CK (U/I) during hot climate season

weeks	T1	T2	T3	SED	P-value
W0	171	240	193	27.10	0.058
W1	124	106	89.3	17.23	0.161
W2	139	94.0	101	33.30	0.356
W3	107	104	95.9	19.54	0.834
W4	131	99.0	105	37.00	0.665
W5	164	109	114	32.70	0.210
W6	175b	79.0a	97.0a	28.50	0.008

T1= treatment one ((no Zinc supplementation) (0 mg Zn/day)), T2= treatment two (36 mg Zn/day), T3= treatment three (72 mg Zn/day), Means with different superscript letters differ (P<0.05). SED=standard error of deviation, Repeated measurements analysis: SED values: T= 10.54, Time= 10.11, T×Time= 19.05 P-values: T= 0.124, Time= 0.001, Time×T=0.014.

Table (7): The effect of zinc (Zn) supplementation on lamb Glucose (mg/dl) during hot climate season

weeks	T1	T2	T3	SED	P-value
W0	29.1	24.6	28.0	3.620	0.436
W1	33.1	31.9	35.1	4.370	0.755
W2	24.2	26.4	28.2	4.060	0.622
W3	51.1	54.2	45.7	5.170	0.265
W4	55.6	60.4	53.3	3.290	0.112
W5	55.1a	68.9b	53.1a	4.650	0.005
W6	53.9	51.0	49.9	4.140	0.614

T1= treatment one ((no Zinc supplementation) (0 mg Zn/day)), T2= treatment two (36 mg Zn/day), T3= treatment three (72 mg Zn/day), Means with different superscript letters differ (P<0.05). SED=standard error of deviation, Repeated measurements analysis: SED values: T= 2.179, Time= 2.355, T×Time= 4.360 P-values: T= 0.389, Time= 0.001, Time×T=0.119.

Table (8): The effect of zinc (Zn) supplementation on lamb T3(ng/ml) during hot climate season

weeks	T1	T2	T3	SED	P-value
W0	2.01	1.97	1.83	0.216	0.688
W1	1.79	1.75	1.93	0.206	0.647
W2	2.33	2.68	2.09	0.232	0.059
W3	2.49b	2.37b	1.81a	0.244	0.024
W4	2.81b	2.43ab	1.86a	0.318	0.024
W5	2.45	1.71	1.68	0.360	0.074
W6	2.21	2.45	1.89	0.314	0.224

T1= treatment one ((no Zinc supplementation) (0 mg Zn/day)), T2= treatment two (36 mg Zn/day), T3= treatment three (72 mg Zn/day), Means with different superscript letters differ (P<0.05). SED=standard error of deviation, Repeated measurements analysis: SED values: T= 0.1384, Time= 0.1491, T×Time= 0.2763 P-values: T= 0.017, Time= 0.003, Time×T=0.045.

Table (9): The effect of zinc (Zn) supplementation on lamb T4 (ng/ml) during hot climate season

weeks	T1	T2	T3	SED	P-value
W0	69.7	73.7	66.0	4.660	0.278
W1	66.7	78.8	71.0	5.820	0.130
W2	80.7	87.4	78.1	7.980	0.497
W3	73.6	82.9	73.3	8.410	0.440
W4	93.6b	76.8a	74.0a	7.100	0.024
W5	76.9	61.6	71.7	8.290	0.197
W6	86.0	78.9	78.7	7.790	0.577

T1= treatment one ((no Zinc supplementation) (0 mg Zn/day)), T2= treatment two (36 mg Zn/day), T3= treatment three (72 mg Zn/day), Means with different superscript letters differ (P<0.05). SED=standard error of deviation, Repeated measurements analysis: SED values: T= 4.638, Time= 3.499, T×Time= 7.279 P-values: T= 0.562, Time= 0.001, Time×T=0.037.

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