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Semen quality, plasma testosterone, and trace element concentrations in response to dietary supplementation of an organic versus an inorganic source of zinc in Mahabadi bucks

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Abstract This study was conducted to evaluate the effects of dietary supplementation of different sources of zinc on the quantitative and qualitative characteristics of semen and blood testosterone in Mahabadi mature bucks. Twenty-four mature male goats (40 ± 0.86 kg), approximately 1-1.5 years old, were divided into three groups (n = 8) and assigned to experimental treatments for 56 days in a completely randomized design. The treatments included: 1. control (a basal diet containing 22.14 mg/kg zinc without zinc supplement), 2. basal diet + 35 mg/kg Zn as ZnSO4 (an inorganic source of Zn), and 3. basal diet + 35 mg/kg Zn as ZnMet (an organic source of Zn). Qualitative (ejaculate volume, sperm concentration) and quantitative (viability, membrane integrity, morphology, and CASA detected parameters) properties of semen and plasma testosterone and Zn, Cu, and Fe concentrations were evaluated. Results showed that ZnMet vs. ZnSO4 increased sperm membrane integrity and plasma Zn concentration and reduced morphologically abnormal spermatozoa (P≤0.05), but there were no significant differences in other parameters. Viability, membrane integrity, morphology, some of the CASA detected parameters (total motility, straight-line velocity, average path velocity, beat cross frequency of sperm), and the plasma concentration of testosterone in zinc-supplemented groups was significantly higher than the control group. In conclusion, the positive effects of an organic source of Zn were seen in reducing abnormal spermatozoa and improving membrane integrity, plasma Zn concentration, and plasma testosterone concentration.

Keywords: reproduction, semen, testosterone, trace elements, zinc

Introduction

Goats are versatile creatures crucial to rural and urban conmmunities' economies and food supplies. The tropics are known for their periodic lack of forage from natural pastures, which has persistently reduced goat industry production, especially for peasant farmers who cannot afford the cost of feed conservation (Ukanwoko et al., 2013). The Iranian rural population relies heavily on goats for their livelihood. In addition to being an essential protein source, goat reproduction improvement can provide income from more

kids and facilitate meeting urgent household needs.

In animal feeding, Zn is one of the most restricted trace minerals due to the body's inability to retain large amounts of it, so it should be included in the diet of ruminants on a daily basis (Suttle, 2010). Zinc significantly contributes to the reproduction of ruminants, and it is essential for the maintenance and process of spermatogenesis and the control of testosterone production, testicular development, sperm maturation, and motility (Liu et al., 2020). Zinc and spermatogenesis have a straightforward relationship, and different parts of the male reproductive system, as well as semen, contain high amounts of zinc (Liu et al., 2015). Zinc deficiency leads to gonadal dysfunction, small size of the testicles, dysfunction in secondary sexual traits, and atrophy of the seminiferous tubules (Hernández-Meléndez et al., 2015). Additionally, zinc regulates the ATP system in spermatozoa, which is vital for sperm motility and alters the patterns of motility and velocity (El-Masry et al., 2010). There is a strong association between the plasma concentration of Zn and plasma testosterone, in which an adequate supply of Zn is vital for sperm function (Liu et al., 2020).

Zinc is crucial for initiating and maintaining spermatogenesis in bucks (Rahman et al., 2014; Narasimhaiah et al., 2018). Supplementing zinc in young bucks increases daily sperm production (Underwood and Somers, 1969; Arangasamy et al., 2018; Narasimhaiah et al., 2018; Liu et al., 2020). Roy et al. (2013) suggested that zinc supplementation could diminish the production of abnormal sperm. Insufficient zinc intake can compromise the DNA regeneration mechanism and make sperm cells susceptible to oxidative damage (Suttle, 2010).

Because of their higher bioavailability and higher absorption rates, organic minerals are more effectively absorbed by the body. Organic mineral supplements have increased the male fertility (Arthington et al., 2002; Kumar et al., 2006; Rowe et al., 2014). Organic zinc supplementation stabilized sperm membranes and reduced oxidative damage in goats (Rahman et al., 2014; Narasimhaiah et al., 2018; Liu et al., 2020) and improved sperm membrane integrity in crossbred bulls (Kumar et al., 2006). Studies in humans, goats, and cattle have shown that chelated zinc is effective in upholding and stabilizing sperm motility and head to tail attachment (Kvist, 1982; Kvist and Björndahl, 1985; Kvist et al., 1987; Kvist et al., 1988; Kumar et al., 2006; Arangasamy et al., 2018).

Majority of Iranian soils are very calcareous and thus cultivated plants are poor in Zn (Ziaeian and Malakouti, 2001); therefore, the diet of ruminants may need to be supplemented with Zn. In addition, different sources of Zn have different bioavailabilities, and there are few studies on the impact of various Zn sources on the reproductive characteristics of bucks. The current experiment aimed to determine how different sources of Zn supplementation affected the qualitative and quantitative traits of semen and blood testosterone in mature Mahabadi bucks in the western region of Iran.

Materials and methods

Feeding management and mineral supplementation

The experiment was carried out during the breeding season following the instructions provided under veterinarian supervision and in accordance with the ethics committee of Bu-Ali Sina University's research policy. Twenty-four healthy Mahabadi breed bucks (body weight of 40 ± 0.86 kg and 1-1.5 years of age) were fed a basal diet formulated in accordance with the National Research Council's recommendations (NRC, 2007). After a 14-day adaptation period, the animals were randomly assigned to three groups (n = 8) for 8 weeks. The treatments included: 1. control (the basal diet contains 22.14 mg/kg zinc without a zinc supplement), 2. basal diet + 35 mg/kg zinc as zinc sulfate, and 3. basal diet + 35 mg/kg zinc as zinc-methionine. Table 1 lists the ingredients and chemical composition of the basal diet. The basal diet (64.16% roughage and 35.84% concentrate mixture) contained 22.14 mg/kg zinc. The bucks were individually housed and received feed at 9:00 am and 5:00 pm for 56 days. The animals were provided with clean and fresh drinking water *ad libitum*. The organic source of zinc (Lee, 2005) was Zn methionine (18% Zn), via a modified process (The United States, patent publication number US7087775 B2). The inorganic source of zinc was analytical grade $ZnSO_4 7H_2O$ (Merck, Germany). Before the morning meal, ZnMet and $ZnSO_4$ were mixed into finely ground barley flour as a carrier for the premix and fed to bucks (the required Zn quantity was adjusted based on dry matter intake). According to AOAC guidelines (AOAC, 2005), samples of dietary components were assessed for dry matter (DM), organic matter (OM), and crude protein (CP) concentration. According to Van Soest et al. (1991), acid detergent fiber (ADF) and neutral detergent fiber (NDF) were measured, and the difference between DM and ash contents was used to estimate the sample organic matter content. The National Research Council's tables (NRC, 2007) were also used to determine the diet metabolizable energy (ME) content.

Semen collection and sperm characteristic analysis

Before starting the trial, bucks were adopted for the collection of semen by the artificial vagina. Three ejaculates in a week, at two-day intervals, were collected from each animal at weeks 4 and 8 of the experiment (24 × 3 = 72 ejaculates per week). The ejaculates, collected by the same trained person, were immediately transferred to a warm water bath at 37˚C and subjected to analysis.

Table 1. Ingredients and nutrient composition of the basal diet (dry matter basis)

Items	Alfalfa hay (60.41%)	Barley straw (3.76%)	Barley (28.79%)	Soybean meal (7.05%)	Basal diet
Dry matter (%DM)	93.87	95.23	93.07	49.49	93.74
Organic matter (% DM)	87.32	89.21	90.26	87.14	88.23
Crude protein (% DM)	12.32	4.41	12.06	44.26	14.20
ME (Mcal/kg)	2.10	1.50	3.00	3.00	2.40
NDF (% DM)	57.23	70.75	25.5	18.2	45.86
ADF (% DM)	35.13	53	6.8	7.3	25.69
Ca (% DM)	1.7	0.06	0.08	0.39	1.08
P (% DM)	0.21	0.07	0.31	0.50	0.25
Zn (mg/kg DM)	22.7	7.15	13.66	60.00	22.14
Cu (mg/kg DM)	13.04	5.20	5.35	19.00	10.95
Fe (mg/kg DM)	302.00	78.26	93.40	185.00	225.31

ME: metabolizable energy (calculated based on NRC, 2007), NDF: neutral detergent fiber, ADF: acid detergent fiber, DM: dry matter, Ca: calcium, P: phosphorus, Zn: zinc, Cu: copper, Fe: iron.

Semen volume

The ejaculate volume (mL) was evaluated in a graduated glass tube (with an accuracy of 0.01 mL) that was connected to the artificial vagina. Each sample was collected in a separate tube.

Sperm concentration

A hemocytometer chamber was used to determine the concentration of sperm after mixing with distilled water to extend the semen (1:200). All measurements were carried out by the same operator using a phase-contrast microscope at 200× magnification. Sperm concentration was expressed as 10^9/mL (Mekasha et al., 2007).

Live spermatozoa and abnormal morphologies

The Eosin-Nigrosin stain was utilized to calculate the proportions of live, dead, and morphologically defective spermatozoa (Swanson and Bearden, 1951; Tomar, 1970). A phase-contrast microscope was used to count a minimum of 200 spermatozoa to examine the percentages of live, dead, and morphologically aberrant spermatozoa at 400x magnification (Labomed LX400 Labo America Inc., Fremont, USA). The sperm containing cytoplasmic droplet, without tail, and with bent tail, shoe hook tail, and coiled tail were considered abnormal.

Sperm membrane integrity

Evaluation of sperm membrane integrity (HOS-G test) was carried out according to the protocol previously described (Revell and Mrode, 1994). The standard method was used for testing raw semen by incubation of 10 µL of semen added to 0.2 mL of hypo-osmotic solution (osmotic strength 125 mOsm kg^{-1}) for 30 min at 37°C and 0.3% glutaraldehyde was added to stop the reaction. The hypo-osmotic solution consisted of 0.9 g fructose and 0.49 g trisodium citrate in 100 mL ddH_2O. A 10 µL sample was applied to a warm and clean coverslip, dried and incubated with buffered formal saline solution for 30 min. Afterward, the slides were washed in distilled water, incubated overnight in Gimsa stain, washed, air dried, and then viewed under a phase contrast microscope at 400× magnification using Labomed LX400 (Labo America Inc., Fremont, USA). Spermatozoa with intact plasma membranes swelled in response to this test, a phenomenon known as tail bending, coiling, or shortening. At least 200 spermatozoa were counted to analyze the proportion of spermatozoa reacting to the HOS-G test; spermatozoa were classified into four subpopulations depending on the swelling pattern: 1. HOS positive and acrosome integrity positive (M+A+), 2. HOS positive and acrosome integrity negative (M+A-), 3. HOS negative and acrosome integrity positive (M-A+) and, 4. HOS negative and acrosome integrity negative (M-A-). The (M+A+) and (M+A-) spermatozoa were added to calculate HOS-positive cells, whereas (M+A+) and (M-A+) were added together to calculate acrosome-positive cells.

Sperm velocity evaluation by computer-assisted sperm analysis (CASA)

The CASA (VideoTesT-Sperm 2.1, Russia) measurements were carried out as previously described (Larsen et al., 2000). Fresh semen (2 µL) was mixed with 500 µL phosphate buffer solution (PBS, pH 7.2), and 5 µL of this mixture were transferred onto a pre-warmed (37°C) microscope slide and placed on the stage of a phase-contrast microscope (Labomed LX400 Labo America Inc., Fremont, USA). Five field images of 25 frames at 10 × magnifications were randomly selected and analyzed (at least 500 cells). The attributes of buck spermatozoa were set and analyzed as follows: Particle size (3-70 m), rapid (>50 m/s), medium motile (>15-45 m/s), slow motile (>10-15 m/s), static (10 m/s), circular (50% linearity, LIN), and progressive forward motility (>80% straightness, STR). The motility parameters, straightness (STR), straight-line velocity (VSL), curvilinear velocity (VCL), linearity (LIN), average path velocity (VAP), and individual progressive motility were analyzed and calculated as described previously

(Palacín et al., 2013). Ten ejaculate per goat were assayed.

Plasma testosterone

Blood samples of all of the bucks were collected by jugular vein puncture (containing heparin) at 9:00 am before the morning meal on days 30 and 56. Plasma samples were separated by centrifugation of blood samples at 1008 g for 20 min, and stored at –80°C in sterilized glass vials. The ELISA method measured plasma testosterone concentration using a commercial kit (AccuBind kits, Monobind, USA, product code: 3725-300) following the manufacturer's instructions. Briefly, 25 µL of plasma was added to a 50 µL testosterone enzyme reagent (mixed for 20-30 seconds), and then a 50 µL testosterone-biotin reagent was added (mixed for 20-30 seconds) to each well. The wells were incubated for 60 minutes at room temperature and 350 µL of wash buffer (containing a surfactant in buffered saline that was diluted with 1000 mL deionized water) were added to the mixture. Afterward, 100 µL of working substrate solution (containing tetramethylbenzidine and hydrogen peroxide in buffer) were added to each wells and incubated at room temperature for 15 minutes, Then, 50 µL of stop solution were added to each well and gently mixed for 15-20 seconds. The absorbance was read at 450 nm in a microplate reader. The intra-assay coefficient of variation for the testosterone assay was less than 5.66%. The ELISA kit had a sensitivity of 0.576 pg/mL.

Plasma Zn, Cu, and Fe

According to Rimbach et al. (1998), plasma was wet digested with 0.3 $_M$ HCl (1:20, v/v), and concentrations of Zn, Cu, and Fe were determined by a flame atomic absorption spectrometry (SpectrAA220 variant, Australia) at a wavelength of 231.9 nm.

Statistical analysis

The data from three ejaculates were averaged as the final results, and semen quality and quantity traits were analyzed by completely randomized design (CRD) using the Proc GLM of the SAS software (Ver. 9.3) using the following model:

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

Blood testosterone concentrations were analyzed as repeated measures in a completely randomized design using the Proc MIXED according to the following statistical model.

$$Y_{ijk} = \mu + T_i + Time_j + T_i \times Time_j + e_{ijk}$$

In these equations, Y_{ijk} is observed value for ith treatment and jth time of measurement in kth replicate; µ overall mean, T_i the effect of the ith treatment; Bx_{ij} coefficient for the variable x_{ij}; $Time_j$ the effect of j th time of measurement; $T_i \times Time_{ij}$ the interaction of ith treatment and jth time of measurement and finally e_{ijk} main error. Duncan's multiple comparisons were used to analyze statistical significance (Duncan, 1955). The difference between treatments was considered significant at the P≤0.05 level. All Data were expressed as mean ± S.E.M.

Results

Semen volume, sperm concentration, sperm viability, and intact acrosome

The effects of zinc sources on ejaculate volume, sperm concentration, sperm viability, and sperm membrane integrity are shown in Table 2. Organic zinc supplementation significantly increased the ejaculate volume, sperm concentration, and sperm number per ejaculate (P≤0.05). Moreover, membrane integrity and viability were significantly higher in the Zn-supplemented groups (organic and inorganic) compared to the control group (P≤0.05).

Computer-assisted semen analysis (CASA) of fresh semen

As shown in Table 2, zinc supplementation caused a significant increase in total sperm motility, straight-line velocity (VSL), velocity in the direct path (VAP), and beat cross frequency (BCF) compared to the control (P≤0.05). However, there was no noticeable variation in other parameters of sperm velocity.

Sperm morphology

According to the data in Table 2, zinc supplementation induced a substantial increase in improving the morphology of all sperm, whose abnormal mid-piece showed a significant decrease in comparison to the control group (P≤0.05). Also, abnormal sperm tails in the treatment group that received ZnMet supplementation were significantly reduced compared to other treatments (P≤0.05). Total morphologically abnormal sperm in the ZnMet group were significantly lower than inorganic zinc and control groups (P≤0.05).

Effect on serum testosterone concentration

As shown in Table 3, plasma testosterone concentration levels were significantly affected by Zn supplementation (P≤0.05), and the highest concentration of testosterone was observed in the ZnMet group.

Effect of Zn supplementation on blood plasma Fe, Cu, and Zn concentrations

The dietary Zn supplementation improved plasma Zn levels on day 56 of the experiment (Table 3, P≤0.05). Goats fed diets supplemented with ZnMet had signify-cantly higher plasma zinc concentrations compared with the $ZnSO_4$ group. Plasma concentrations of Cu and Fe were not affected by treatments.

Table 2. Effect of dietary supplementation with different sources of zinc (organic vs. inorganic) on semen characteristics in Mahabadi bucks

Item	Treatments			SEM	P values
	Control	$ZnSO_4$	ZnMet		
Ejaculate volume (mL)	0.84 [a]	0.93 [ab]	1.07 [b]	0.05	0.02
Sperm concentration (×10^9/mL)	2.69 [a]	3.24 [ab]	3.55 [b]	0.20	0.02
TSE (×10^9)	2.26 [a]	3.01 [b]	3.80 [c]	0.23	≤0.01
Viability (%)	87.77 [a]	92.44 [b]	95.32 [c]	0.76	≤0.01
Membrane integrity (%)	74.45 [a]	83.05 [b]	88.94 [c]	1.30	≤0.01
Total abnormality (%)	9.51 [a]	6.89 [b]	5.06 [c]	0.38	≤0.01
Abnormal head	2.37	1.52	0.82	0.40	0.57
Abnormal mid-piece	3.11 [a]	2.09 [b]	1.56 [b]	0.22	≤0.01
Abnormal tail	4.01 [a]	3.29 [ab]	2.68 [b]	0.30	0.02
SC (cm)	27.42	27.93	27.83	0.26	0.27
TM (%)	84.75 [a]	88.50 [b]	89.63 [b]	1.18	≤0.02
PM (%)	53.63	56.00	57.63	2.84	0.61
LIN (%)	0.39	0.45	0.45	0.20	0.10
VSL (µm/s)	53.92 [a]	69.38 [b]	67.22 [b]	2.89	0.02
VCL (µm/s)	105.76	122.34	121.25	5.30	0.06
VAP (µm/s)	65.41 [a]	86.83 [b]	84.04 [b]	4.14	≤0.01
ALH (%)	2.70	2.31	2.43	0.15	0.24
STR (%)	0.64	0.65	0.66	0.01	0.83
BCF (Hz)	9.26 [a]	7.58 [b]	7.26 [b]	0.25	≤0.01

SEM: standard error of the mean, TSE: total sperm per ejaculate, SC: Scrotal circumference, TM: total motility, PM: progressive motility, LIN: linearity, VSL: straight-line velocity, VCL: curvilinear velocity, VAP: average path velocity, ALH: amplitude of lateral head displacement, STR: straightness, BCF: beat cross frequency. a,b,c: Within rows, mean values with common superscript (s) are not different (P>0.05; Duncan's multiple range test).

Table 3. Effect of dietary supplementation of different sources with zinc (organic vs. inorganic) on plasma testosterone (TES) and some trace mineral concentrations in Mahabadi bucks

	TES (ng/mL)	Zn (mg/kg)	Cu (mg/kg)	Fe (mg/kg)
Treatments				
Control	1.72 [a]	0.76 [a]	0.98	1.13
$ZnSO_4$	1.89 [b]	0.81 [b]	0.95	1.51
ZnMet	1.92 [b]	0.86 [c]	0.93	1.40
SEM	0.19	0.01	0.02	0.14
Day				
30	1.46 [a]	0.791	0.950	1.35
56	2.23 [b]	0.823	0.964	1.34
SEM	0.159	0.01	0.01	0.11
P-Value				
Treatment	0.05	≤0.01	0.18	0.16
Day	≤0.01	0.06	0.52	0.96
Treatment × Day	0.55	0.40	0.92	0.84

The value of testosterone concentration in plasma on days 30 and 56 is presented as mean ± SEM. a,b,c: Within columns and as per section, mean values with common superscript (s) are not different (P>0.05; Duncan's multiple range test).

Discussion

Organic zinc supplementation had a significant effect on ejaculate volume, sperm number per ejaculate, sperm concentration, and blood testosterone concentration. Similar results were reported in bulls (Kumar et al., 2006) and different buck breeds (Rahman et al., 2014; Arangasamy et al., 2018; Liu et al., 2020). Data on ejaculate volume are consistent with prior studies (Kumar et al., 2006; Narasimhaiah et al., 2018; Liu et al., 2020). Other factors that can be considered are the duration of zinc supplementation and the breeding season (Arangasamy et al., 2018). It should be noted that the average duration of sperm production in bucks is about 47.7 days, which is equivalent to 4.5 epithelial cycles in seminiferous tubules (França et al., 1999). Therefore, this experiment was designed to cover a complete sperm production cycle in goat testis. Several studies have shown that increasing the duration of the experiment, consequently, and the number of sperm-producing cycles results in altering the concentration of metabolic compounds in the body, especially compounds that are effective over time and will improve reproductive performance (Kumar et al., 2006; Imam et al., 2009; Rahman et al., 2014; Hernández-Meléndez et al., 2015; Arangasamy et al., 2018; Narasimhaiah et al., 2018; Liu et al., 2020).

According to Table 2, zinc supplementation (organic and inorganic) caused a significant increase in live sperm (P≤0.05). Our result agrees with some studies on bucks (Rahman et al., 2014; Arangasamy et al., 2018; Narasimhaiah et al., 2018) and bulls (Kumar et al., 2006), who reported an increase in the proportion of viable sperm. The higher sperm viability in the Zn-supplemented groups in this study might be related to its membrane-stabilizing properties associated with protective effects against oxidative damage (Arangasamy et al., 2018). Zinc is also involved in many enzymatic activities and, due to its metalloenzymatic properties, is closely linked to carbohydrate, lipid, nucleic acid, and protein metabolism. Zinc increases the survival

and functional capability of sperm by stabilizing the lysosomes, ribosomes, RNA, and DNA (Bettger and O'Dell, 1981; Kumar et al., 2006; Rahman et al., 2014; Narasimhaiah et al., 2018).

The percentage of functional membrane integrity was significantly (P≤0.05) improved in treatments that received zinc supplements. The results are consistent with previous reports on bucks and crossbred bulls (Kumar et al., 2006; Arangasamy et al., 2018). Organic minerals were discovered to be more effectively utilized in the body due to higher bioavailability and absorption rates (Arthington et al., 2002; Xiaoming et al., 2006). Zinc is notably high in sperm cell membrane fractions and lipoproteins. It stabilizes membranes by forming stable mercaptides from reactions with the sulfhydryl groups of membrane proteins by preventing lipid peroxidation by obstructing phospholipases (Bettger and O'Dell, 1981). Various proteins are found in the secretions of the male accessory glands, and these proteins are crucial for preserving a healthy environment for buffering and safeguarding the shape and viscosity of sperm cells (Patricio et al., 2015). The theory is that supplementation of organic Zn, due to its higher absorption and retention attributes, has improved the accessory gland secretions and ultimately made the sperm structure more stable (Narasimhaiah et al., 2018).

Our findings in velocity parameters (beat cross frequency, straight-line velocity, and average path velocity) and sperm motility (total motility) agree with the previous investigations in crossbred cattle and goats (Kumar et al., 2006; Arangasamy et al., 2018; Narasimhaiah et al., 2018). Supplementing crossbred bulls with Zn (organic or inorganic) enhanced fertility and motility of sperm, and these traits were significantly higher in organic Zn-supplemented bulls compared to inorganic zinc-supplemented (Kumar et al., 2006; Geary et al., 2016). In similar research, feeding of 40 mg/kg organic zinc compared to 20 mg/kg organic zinc increased sperm motility in bucks (Arangasamy et al., 2018; Narasimhaiah et al., 2018). Sperm motility is determined by the amount of available ATP as a source of energy. According to reports, Zn affects the energy-utilization process via the ATP system and regulates the enzymes sorbitol dehydrogenase and lactate dehydrogenase, both of which are crucial for sperm motility (El-Masry et al., 2010). Previous investigations confirmed that Zn contributes to chromatin stability, condensation of chromatin, and head-tail dissociation (Kvist, 1982; Björndahl and Kvist, 1982). Additionally, Zn regulates the motility of sperm in goats by affecting the sperm flagellum development (Saleh et al., 1992). Based on its localization in the central part of the sperm and interaction with the lipoprotein fraction, zinc is implicated in the breakdown of lipids, which is the key source of energy needed for the movement of spermatozoa (Saleh et al., 1992).

Zinc supplementation caused a significant increase in improving the morphological attributes of sperm compared to the control treatment (P≤0.05). The results confirmed earlier reports in sheep and buffalo bulls (Underwood and Somers, 1969; Abdel-Khalek et al., 2010). Sperm cell plasma membranes have a noticeably different lipid content than the somatic cells, including more polyunsaturated fatty acids that inhibit lipid peroxidation damage of spermatozoa, giving them unique structure and function. Lipid peroxidation of the plasma membrane the lipid matrix to disintegrate (Liu et al., 2020). Ultimately, sperm activity is hindered by oxidative stress and the generation of cytotoxic aldehyde (Aitken and Baker, 2004). The improved semen traits were associated with the improved activity of antioxidant enzymes, which increased the defense mechanisms that prevent reactive oxygen species (ROS) generation and boosted the functional properties of the sperm. According to the data obtained in the present study, ZnMet supplementation improved sperm membrane integrity and plasma Zn concentration, which could have reduced the sperm abnormality.

The concentration of plasma zinc was significantly (P≤0.05) increased by Zn supplementation, which is in agreement with the previous reports on goats (Puchala et al., 1999; Kumar et al., 2013; Hernández-Meléndez et al., 2015; Liu et al., 2015; Liu et al., 2020).

Zinc has specific effects on the secretion of testosterone that enhances the characteristics of testicular function and development (Martin et al., 1994). As indicated in Table 3, the mean blood testosterone concentration ($ng.mL^{-1}$) in the Zn-supplemented treatments was significantly higher (P≤0.05) than in the control group. This is in agreement with earlier findings in bulls and different goat breeds (Kumar et al., 2006; Liu et al., 2015; Arangasamy et al., 2018; Mousavi Esfiokhi et al., 2023). Testosterone concentration in different breeds of male goats varies between 0.5 to 7.15 $ng.mL^{-1}$ in the breeding season (Arangasamy et al., 2018; Krishnaiah et al., 2019; Mousavi Esfiokhi et al., 2023). Previous research showed that the testosterone level in response to Zn supplementation is dose-dependent and gradually increases over time (Arangasamy et al., 2018). The duration of Zn feeding and the zinc concentration level in the basal diet are the most critical factors affecting the concentration of testosterone in the blood because feeding minerals is related to a gradual increase in testosterone hormone (Kumar et al., 2006; Arangasamy et al., 2018). Based on the results of the present investigation, it seems that the amount of zinc in the basal diet (22.14 mg/kg) was insufficient for the adequate synthesis of testosterone in the control group. It also appears that feeding zinc supplements for 50 days is sufficient to influence testosterone production and secretion. Moreover, after 30 days, plasma testosterone concentration was increased ($0.77\ ng.mL^{-1}$). This increase in plasma testosterone concentration is possibly due to a phenomenon called the "buck-to-buck effect"; that sexually active bucks are able to stimulate LH and testosterone secretion and improve the sexual behavior of other bucks (Delgadillo et al., 2022).

Conclusion

The quantitative (semen volume, concentration of sperm, and total sperm per ejaculate) and qualitative (increased viability, motility of sperm, acrosome integrity, and plasma membrane) properties of Mahabadi buck semen were enhanced by dietary supplementation with organic or inorganic Zn, which may be beneficial to goat buck fertility.

Declaration of interest

The authors declare that they have no competing interests.

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