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The Libido, Scrotal Circumference, Sperm Quality, and Testosterone Levels of Matured Boer Bucks Supplemented with Selenium

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ABSTRACT

A study was conducted to determine the effect of three levels of selenium (Se) supplementation on the libido, scrotal circumference, sperm quality, and testosterone levels in matured Boer bucks. The feeding trial was conducted at MARDI Bachok Station, Bachok, Kelantan, Malaysia, for over six months. A total of 18 matured Boer bucks aged two years with an initial body weight of 53.28±0.62 kg, and no experience of sexual activity was selected for the feeding trial. All animals were randomly divided into three dietary treatments based on a completely randomized design (CRD). Group A and B were supplemented with organic Se at doses of 0.18 mg/kg DM and 0.36 mg/kg DM, respectively, whereas group C acted as a control group (without Se supplementation). Their basal diet consisted of 60% formulated pellet and 40% *Brachiaria dictyoneura* **grass. Daily feed offered (DM basis) was based on 3% of mean body weight. Libido, scrotal circumference, and sperm quality were measured every two months during the feeding trial. Testosterone levels were determined at the end of the feeding trial. The results indicated that the basal diet (control group) was deficient in Se, with the concentration of 0.004 mg/kg. Supplementation of 0.18 mg Se/kg DM only improved the libido and sperm quality ($p<0.05$). Supplementation of 0.36 mg Se/kg DM significantly ($p<0.05$) increased libido, sperm quality, and testosterone levels. It is summarized that supplementation of 0.36 mg Se/kg DM has the potential to improve the reproductive performance of bucks, although it occurred at the adult stage.**

Keywords: organic selenium; matured Boer bucks; libido; sperm quality

INTRODUCTION

Boer goats have been popularly raised in Malaysia for meat production (Ali *et al.*, 2012). Boer was imported to Malaysia in 2000 to increase local goat production, including those from South Africa and Australia (Rosali *et al.*, 2019; Sabri *et al.*, 2013). The feeding system for goats, including Boer, mainly depends on locally available forage and concentrate mixtures (Abdullah *et al.*, 2015; Mohd Noor *et al.*, 2020). These feed resources are often inadequate in nutritive values, with crude protein (CP) content less than 6%, fiber content of more than 25%, and a lack of minerals and vitamins (Ali *et al.*, 2012). Nutrient imbalances and deficiencies can cause various problems in goat production, which are mainly associated with slow growth performance, high parasite infestation, low milk production, and low rate of reproduction (Abdullah *et al.*, 2015; Mira *et al.*, 2018; Mohd Noor *et al.*, 2020).

Low libido is one of the cases associated with reproductive inefficiency in bucks caused by nutrient imbalances and deficiencies (Abdullah *et al.*, 2015; Ahsan *et al.*, 2014). In local Boer bucks, cases of low libido were reported (Rosali *et al.*, 2019; Mariani *et al.*, 2011). Mariani *et al.* (2011) reported that the local Boer bucks showed low libido in their initial study, with the reaction time of the first mount being 106.60 sec. The reaction time of more than 65 sec was generally regarded as a low libido buck (Ford *et al.*, 2009). Rosali *et al.* (2019) also reported that local Boer bucks had a problem expressing their reproductive potential compared to the Boer does. In Indonesia, 7 out of 20 heads of Boer bucks were also found with low libido and poor semen quality (Suyadi, 2012). These cases agree with those reported by Ford *et al.* (2009). They demonstrated that matured Boer bucks appeared to be less sexual interest in females and had lower libido than the Kiko bucks (Feral crossbred and originated from New Zealand). No studies have been done to identify the cause of low libido in matured Boer

bucks and rectify this problem. This study will help understand the importance of selenium (Se) supplementation in enhancing the reproductive performance of matured bucks. When that problem is rectified, the poor reproductive performance of matured Boer bucks can be marketed as a quality breeder, maximizing farmers' income and farm productivity.

Nutritional manipulation from trace minerals significantly influences animal reproduction (Schweinzer *et al.*, 2017). Selenium has been reported to play an essential role in animal reproduction (Bano *et al.*, 2018). The deficiency of Se is affecting reproductive performance (Haenlein & Anke, 2011). Selenium deficiency has been reported to cause infertility, lower motility sperm, and a higher percentage of sperm abnormality in various species (Ahsan *et al.*, 2014). Selenium supplementation had a significant role in influencing male fertility, including male hormone (testosterone), sperm quality, testis, and libido, especially in the area that had Se deficiency (Ahsan *et al.*, 2014; Bano *et al.*, 2018).

Forages and soils are two decisive factors affecting Se deficiency (Ahsan *et al.*, 2014; Sayiner & Karagul, 2017). Selenium concentrations of less than 0.5 mg/kg in the soil and less than 0.1 mg/kg in plants are considered Se deficiency (Ramirez *et al.*, 2004). Rainfall, evaporation, and pH levels affect Se concentration in the soil (Ahsan *et al.*, 2014; Sayiner & Karagul, 2017). In Malaysia, high levels of rainfall and heavy leaching (Ali *et al.*, 2012) could cause Se deficiency in both forages and soils, which are also attributable to Se deficiency, particularly in ruminant livestock (Zubair *et al.*, 2015). As low libido is often associated with Se deficiency (Mehdi & Dufrasne, 2016), studies are required to determine the effect of three levels of Se supplementation on the libido, scrotal circumference, sperm quality, and testosterone levels in matured Boer bucks.

MATERIALS AND METHODS

Animals

A six-month (180 d) feeding trial was conducted at the Boer goat farms, MARDI Bachok Station, Bachok, Kelantan, Malaysia. This study was approved by the Universiti Malaysia Kelantan Animal Ethics Committee (UMK/FPV/ACUE/RES/4/2020). The feeding trial was conducted in the rainy season (July to December). A total of 18 matured Boer bucks, two years of age, with no previous experience of sexual activity, were selected. They did not have previous experience of sexual activity because they were separated from the female after they reached the weaning age of 3 months. Male and female goats were separated to control production as the farm was for research only.

The bucks were divided into three different dietary treatments, with six bucks for each group using a completely randomized design (CRD). Their initial body weight means was 53.28±0.62 kg (SEM). The bucks were housed separately in an individual pen of 4 x 3 feet in dimension, equipped with an individual feeder. They were also dewormed with fenbendazole before the start of the feeding trial.

Experimental Treatments

The dietary treatments were divided into three groups, namely group A, group B, and group C. Organic Se at the levels of 0.18 mg/kg DM and 0.36 mg/kg DM were added in the mixture of rations for groups A and B, respectively, and pelleted (Lukusa & Lehloenya, 2017). Group C acted as a control group whereby no Se supplementation was added. Se supplementation in group A was within the Se requirement of 0.10-0.30 mg/kg DM for goats (NRC, 2007), while Se added in group B was twice the level of Se in group A and more than the maximum requirement for goats (0.30 mg/kg DM) (NRC, 2007). The three levels of Se were evaluated to determine the appropriate level of Se that can increase the reproductive performances of local Boer bucks.

The basal diet consisted of 60% formulated pellet and 40% *Brachiaria dictyoneura* grass, following the guideline Jolly (2013) reported. Se supplementation was mixed with the formulated pellet. Feed offered (basal diet) was calculated based on 3% of mean initial body weight on a DM basis. The bucks were fed formulated pellets at 9:00 am to ensure they received their Se supplementation. The bucks were fed *B. dictyoneura* grass in the evening at approximately 3:00 pm. Clean and fresh water were provided *ad libitum* throughout the feeding trial.

Preparation of Formulated Pellet and *Brachiaria dictyoneura* Grass

The organic Se in the form of Se-enriched yeast powder (Selemax® 2000, Brazil) that contained 2000 mg Se/kg was used in this study. It was then mixed with ground corn at a 8.40% inclusion level before undergoing the pelleting process. The ground corn was used as a carrier to ensure Se was well mixed in the feed mixture (Qin *et al.*, 2011). The formulated pellets were prepared at the IA Agro feed mill, Melor, Kelantan, Malaysia. The

Table 1. Ingredients in the dietary treatments of matured Boer bucks

Variables (% DM)	Dietary treatments		
	Group A	Group B	Group C
Brachiaria dictyoneura grass	40.00	40.00	40.00
Palm kernel cake (PKC)	20.40	20.40	20.40
Rice straw	18.00	18.00	18.00
Ground corn	8.40	8.40	8.40
Soya bean meal	7.80	7.80	7.80
Di-Calcium phosphate (DCP)	0.90	0.90	0.90
Salt	0.30	0.30	0.30
Molasses	3.00	3.00	3.00
Limestone	0.60	0.60	0.60
Ammonium chloride	0.30	0.30	0.30
Mineral vitamin premix	0.30	0.30	0.30
Selenium (mg/kg DM)	0.18	0.36	-

Note: Group A= Supplemented with 0.18 mg organic Se/kg DM, Group B= Supplemented with 0.36 mg organic Se/kg DM, Group C= No Se supplementation (control). The total ratio in the dietary treatments is 60%: 40% (formulated pellet: grass).

ingredients in the dietary treatments for group A, group B, and group C are shown in Table 1.

B. dictyoneura grass at six weeks of age was used as the main roughage. The grass was cut at approximately 10:00 am daily. The cutting was carried out by a grass cutter 10 cm above the soil surface. The grass was collected, weighed, and fed to the bucks at 3.00 pm. Samples in triplicate from the formulated pellets (group A, group B, and group C) and grass were collected monthly. The chemical compositions of dietary treatments are shown in Table 2.

Chemical Analysis for the Dietary Treatments

Analysis of formulated pellets and *B. dictyoneura* grass for DM, ash, CP, and EE were conducted using AOAC (2012) procedures. While analysis of CF, ADF, and NDF was determined as described by Dewi *et al.* (2018). Mineral P, Ca, Cu, and Se was analyzed based on the standard analytical method for Inductively Coupled Plasma Mass Spectrometry (ICP-MS, Perkin Elmer Corporation, USA) (Aghwan *et al*., 2016). The chemical analysis was carried out at the Agriculture Chemical Laboratory MARDI, Serdang, Selangor, Malaysia.

Libido and Scrotal Circumference

The experimental buck was placed at the start point for the libido test, and it was three meters away from a restrained oestrus female in the test pen. The buck at the start point was released and allowed to mate the restrained oestrus female in 15 minutes (Ángel-García *et al*., 2015). Oestrus on the female was treated using the CIDR specific for goat (Eazi-Breed CIDR®, Pfizer, New Zealand). Reaction time in second was measured using a stopwatch (Suyadi, 2012). Reaction time was recorded from the buck released until the first mount attempt, and the buck showed the penis erected (Ángel-García *et al*., 2015; Suyadi, 2012). Scrotal circumference in cm was measured using a flexible tape at the widest scrotal diameter (Lukusa & Lehloenya, 2017). Libido and scrotal circumference were measured in the morning by the 2-month interval throughout the feeding trial.

Table 2. Chemical compositions of dietary treatments of matured Boer bucks (±SEM)

Variables	Dietary treatments		
	Group A	Group B	Group C
Nutrient (%)			
DM	66.25±0.05	66.25±0.05	66.25±0.05
Ash	6.78±0.11	6.78±0.11	6.78±0.11
CP	11.68±0.21	11.68±0.21	11.68±0.21
EE	3.45±0.07	3.45±0.07	3.45±0.07
CF	22.42±0.53	22.42±0.53	22.42±0.53
ADF	31.42±0.60	31.42±0.60	31.42±0.60
NDF	58.26±0.43	58.26±0.43	58.26±0.43
Mineral			
Ca (%)	0.53±0.03	0.53±0.03	0.53±0.03
P (%)	0.27±0.01	0.27±0.01	0.27±0.01
Cu (mg/kg)	0.10±0.08	0.10±0.08	0.10±0.08
Se (mg/kg)	0.18±0.01	0.36±0.01	0.004±0.00
Ca:P	2:1	2:1	2:1
Calculated ME (MJ/kg DM)	7.85±0.03	8.10±0.03	7.70±0.11

Note: SEM= Standard error of the mean. DM= Dry matter, CP= Crude protein, EE= Ether extract, CF=Crude fiber, ADF= Acid detergent fiber, NDF= Neutral detergent fiber, P= Phosphorus, Ca= Calcium, Cu= Copper, Se= Selenium, ME= Metabolizable energy. Group A= Supplemented with 0.18 mg organic Se/kg DM, Group B= Supplemented with 0.36 mg organic Se/kg DM, Group C= No Se supplementation (control). The total ratio in the dietary treatments is 60%: 40% (formulated pellet: grass).

Sperm Quality

Before starting the experiment, semen collection training using an artificial vagina (AV) on the bucks was performed twice per week for one month. Then, a 2-month interval was conducted to evaluate the sperm quality of the bucks. As the formation of mature sperms (spermatogenesis) was reported to take approximately 50 days (Franca *et al*., 1999), the duration of the study was considered adequate to ensure the effectiveness of Se supplementation on the sperm quality of Boer bucks. Fresh semen samples were collected from each buck using an AV in the morning, as Suyadi (2012) described. Immediately after collection, the tubes with fresh semen were transported to the laboratory in an insulated container containing distilled water at 37 ^{o}C. At the laboratory, all samples were transferred in a water bath at 37 ^{o}C. The volume of semen was recorded using a 15 mL graduated tube. Fresh semen was evaluated under a light microscope for sperm concentration, sperm motility, sperm viability, and sperm abnormality (CX21, Olympus, Japan). Sperm quality was determined by two experienced evaluators, and the mean was calculated.

Sperm concentration was determined using the Makler counting chamber (Sefi- Medical Instruments) (Rai *et al*., 2017). A drop of fresh semen was diluted with ten drops of phosphate buffer saline (PBS, 10X). Diluted semen was put on the Makler counting chamber and allowed to rest for 5 min. Sperms in 30 squares of the counting chamber were counted under 200X magnification of the light microscope. Sperms with their heads or only heads present in the 30 squares were counted. Sperm concentration (number per milliliter) was determined by counting the sperms in 30 squares and dividing the total sperms by three, then multiplied by 10^6 and by the dilution factor (10X).

Sperm motility was determined using the Makler counting chamber (Rai *et al*., 2017). A drop of fresh semen was diluted with ten drops of phosphate buffer saline (PBS, 10X). Diluted semen was then dropped on the chamber and rested for 5 min. The moving sperm was viewed under 200X magnification of the light microscope. All sperms were counted in 16 squares. Then, the moving sperm in those squares were counted. The percentage of sperm motility was calculated as total moving sperms in the 16 squares divided by the total count of sperms in the 16 squares, then multiplied by 100.

Sperm viability was determined according to Lukusa & Lehloenya (2017). A drop of diluted semen

was mixed with a drop of eosin-nigrosine stain at the edge of a glass slide. Another glass slide was used to make a thin smear. Then, the smear was allowed to dry. A total of 100 sperms were viewed under 400X magnification of the light microscope from 4 microscopic fields. Sperms that take the stain resulting in purple or redheads were recorded as dead sperms. While those sperm that did not take the stain were recorded as live sperm when their heads were white. The percentage of sperm viability was calculated as total live sperms divided by the total count of sperms (100 sperms), then multiplied by 100.

Sperm abnormality was determined by using slides as prepared for sperm viability (Lukusa & Lehloenya, 2017). One hundred sperms were viewed under 400X light microscope magnification from four microscopic fields. The percentage of sperm abnormality was calculated as total abnormal sperms divided by the total count of sperms (100 sperms), then multiplied by 100.

Testosterone Levels

At the end of the feeding trial, blood samples for assessing serum testosterone levels were collected from the jugular vein of all bucks. The blood samples were taken using a 21-gauge (1 ½") vacutainer needle (Becton, Dickinson and Company©, USA) into 10 ml plain tubes (BD Vacutainer® red top, USA). The blood was stored at room temperature for 2 to 3 h, later allowed to stand overnight at 4 °C, and was centrifuged at 1500 rpm for 15 minutes. The serum was stored at -20 °C until it was analyzed. The testosterone level was determined using the ELISA kit for the goat (Cusabio, USA). The microplate reader (Asys UVM340, Biochrom, UK) was used to read the optical density (OD) value of each well set at 450 nm wavelength. The standard curve was created using Software RIDASOFT®Win version 1.9 (2015) (R-Biopharm AG, Germany). The software will then calculate the testosterone levels of samples based on the standard curve equation and the coefficient of variation. If the coefficient of variation was more than 15%, the sample was assayed again (Suyadi, 2012).

Statistical Analysis

All data were analyzed using the General Linear Model (GLM) procedure of IBM SPSS Statistics version 26 (2019) at a 95% confidence interval. A two-way Analysis of Variance (ANOVA) with the main factors of dietary treatments (0 mg/kg DM, 0.18 mg/kg DM, 0.36 mg/kg DM), time (months 2, 4, 6), and their interactions were used to analyze the data of libido, scrotal circumference, and sperm quality. Meanwhile, a one-way Analysis of Variance (ANOVA) was used to analyze the data by the 2-month interval of libido, scrotal circumference, sperm quality, and testosterone levels (Figure 1 to Figure 8). When the data had significant differences ($p<0.05$), the data were compared using Duncan Multiple Range Test. The relationship between scrotal circumference with libido and sperm quality of Boer bucks was analyzed using Pearson correlation at a 99% confidence interval.

RESULTS

Se Content in the Dietary Treatments

Se contents in the dietary treatments for group A, group B, and group C were 0.18 mg/kg, 0.36 mg/kg, and 0.004 mg/kg, respectively (Table 2).

Libido and Scrotal Circumference

At the initial (month 0), there was no significant difference in libido between the Se treatments (Figure 1). The initial libidos (sec) of group A, group B, and group C were 70.00, 68.50, and 66.83, respectively. There were significant differences ($p<0.05$) in the effects of dietary treatments on libidos at month 2, month 4, and month 6 of the feeding trial (Figure 1). The Se treatments and time (month) significantly affected ($p<0.05$) the libido of matured Boer bucks (Table 3). However, no interaction between Se treatments and time (month) was found on the libidos of mature Boer bucks. Group A and group B had a higher libido ($p<0.05$) as compared to group C (control group) (Table 3).

No significant difference was found in the effects of dietary treatments on the scrotal circumference at month 0, month 2, month 4, and month 6 of the feeding trial (Figure 2). Table 3 shows the effect of dietary treatments, time, and their interaction on the scrotal circumference. The dietary treatments did not affect the scrotal circumference. However, time (month) significantly affected ($p<0.05$) the scrotal circumference of matured Boer bucks. No interaction between dietary treatments and time (month) was found on the scrotal circumference.

Sperm Quality

At the initial (month 0), there was no significant difference in the semen volume, sperm concentration, sperm motility, sperm viability, and sperm abnormality between the dietary treatments (Figure 3 to Figure 7). There were significant differences ($p<0.05$) in the semen volume, sperm concentration, sperm motility, sperm viability, and sperm abnormality between the Se treatments at month 2, month 4, and month 6 of the feeding trial (Figure 3 to Figure 7). Table 4 shows the effect of dietary treatments, time, and their interaction on sperm quality. The dietary treatments significantly affected ($p<0.05$) the semen volume, sperm concentration, sperm motility, sperm viability, and sperm abnormality. Time (month) significantly affected ($p<0.05$) the sperm concentration, sperm motility, sperm viability, and sperm abnormality except for the semen volume. No interaction between dietary treatments and time (month) was found on the semen volume, sperm concentration, sperm motility, and sperm viability except for the sperm abnormality ($p<0.05$). Group A and group B had high qualities of sperms ($p<0.05$) as compared to group C (control group).

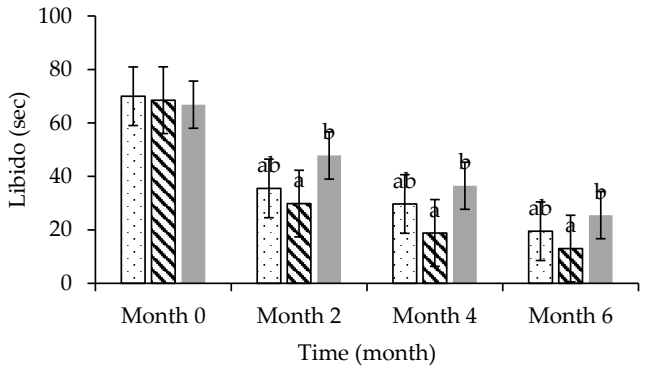

Figure 1. Libido (sec) in matured Boer bucks fed with the dietary treatments at month 0, month 2, month 4, and month 6 of the feeding trial (±SEM). Note: [a,b]Superscript are significantly different ($p<0.05$) in the bars. SEM= Standard error of the mean. Group A= Supplemented with 0.18 mg organic Se/kg DM; Group B= Supplemented with 0.36 mg organic Se/kg DM; Group C= No Se supplementation (control). The number of replicates for each treatment was n=6.
□ Group A ▨ Group B ■ Group C

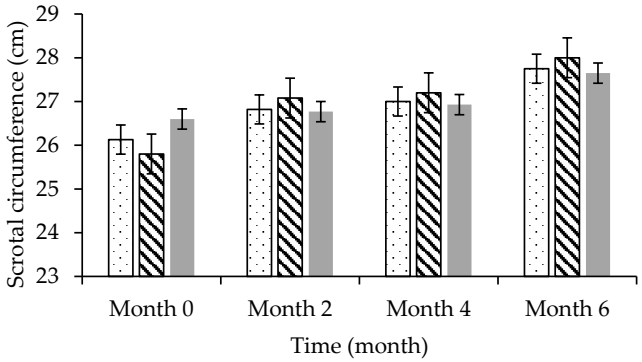

Figure 2. Scrotal circumference (cm) in matured Boer bucks fed with the dietary treatments at month 0, month 2, month 4, and month 6 of the feeding trial (±SEM). Note: SEM= Standard error of the mean. Group A= Supplemented with 0.18 mg organic Se/kg DM; Group B= Supplemented with 0.36 mg organic Se/kg DM; Group C= No Se supplementation (control). The number of replicates for each treatment was n=6.
□ Group A ▨ Group B ■ Group C

Table 3. Libido (sec) and scrotal circumference (cm) of matured Boer bucks fed with the dietary treatments (±SEM)

	Variables	
	Libido (sec)	Scrotal circumference (cm)
Dietary treatments		
Group A	28.22±3.64[b]	27.19±0.16
Group B	20.56±2.44[a]	27.43±0.24
Group C	36.61±2.77[c]	27.12±0.26
P-value		
Treatment	<0.01	0.60
Time	<0.01	<0.01
Treatment*Time	0.90	1.00

Note: [a,b,c]superscript are significantly different ($p<0.05$) in the same column; SEM= Standard error of the mean. Group A= Supplemented with 0.18 mg organic Se/kg DM; Group B= Supplemented with 0.36 mg organic Se/kg DM; Group C= No Se supplementation (control). The number of replicates (each parameter) for Se treatments and time, respectively n=18.

Correlation Between Scrotal Circumference with Libido and Sperm Quality

The correlation analysis between scrotal circumference with libido and sperm quality of matured Boer bucks is shown in Table 5. All parameters had a positive and negative correlation ($p<0.01$) between each other, excluding scrotal circumference and sperm concentration, where no correlation existed between them ($r= 0.21$, $p>0.05$).

Testosterone Levels

Figure 8 shows the testosterone levels among dietary treatments at the end of the feeding trial. The testosterone levels (ng/mL) in group B were significantly higher ($p<0.05$) as compared to group A and group C (control group).

DISCUSSION

Se Content in the Dietary Treatments

The basal diet (control group) in this study was deficient in Se. The amount of less than 0.1 mg Se/kg in roughage and concentrate is considered Se deficiency (Ramirez *et al.*, 2004). Therefore, it is essential to include Se in feed resources (deficient in Se) to enhance the reproductive performance of local Boer bucks.

Libido and Scrotal Circumference

At the initial, Boer bucks showed low libido, which was more than 65 sec (Ford *et al.*, 2009). This finding was in agreement with Rosali *et al.* (2019) and Mariani *et al.* (2011), who reported that the local Boer bucks had low libido associated with Se deficiency. In the present study, the libido of Boer bucks was affected by Se supplementation at 0.18 mg/kg DM and 0.36 mg/kg DM ($p<0.05$) compared to the control bucks without Se supplementation.

Higher libido in the bucks supplemented with 0.36 mg Se/kg DM could be due to the antioxidant effect of Se in protecting the male reproductive tract (Lukusa & Lehloenya, 2017), leading to an increase ($p<0.05$) in the testosterone levels (Figure 8). Increased testosterone levels were reported to increase libido (Hafizuddin *et al.*, 2020; Elbaz & Abdel Razek, 2019). In this study, scrotal circumference correlated with libido ($r= -0.37$, $p<0.01$). Thus, the scrotal circumference of more than 25 cm in this study will produce higher testosterone levels, leading to an increase in libido (Tibary *et al.*, 2018). Experience and learning processes were reported to increase the mating ability of bucks (Mariani *et al.*, 2011), suggesting that libido is also affected by time.

Information concerning the effect of Se supplementation on the libido of bucks is not available on other animals (Ahsan *et al.*, 2014). In rams, libido had significantly improved when the rams were fed with Se at 0.10

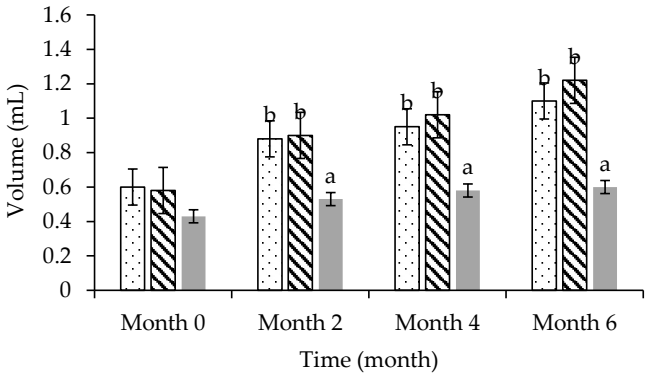

Figure 3. Semen volume (mL) in matured Boer bucks fed with the dietary treatments at month 0, month 2, month 4, and month 6 of the feeding trial (±SEM). Note: [a,b]Superscript are significantly different (p<0.05) in the bars. SEM= Standard error of the mean. Group A= Supplemented with 0.18 mg organic Se/kg DM; Group B= Supplemented with 0.36 mg organic Se/kg DM; Group C= No Se supplementation (control). The number of replicates for each treatment was n=6.
□ Group A ▨ Group B ■ Group C

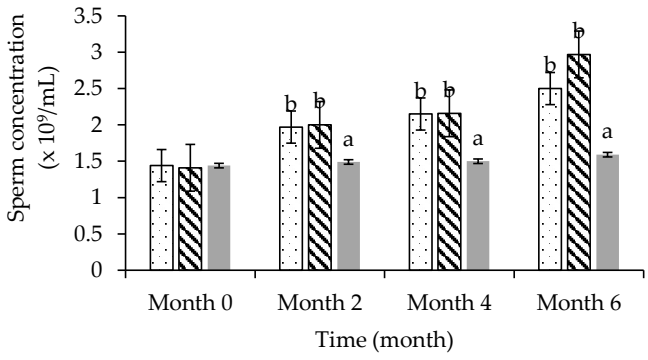

Figure 4. Sperm concentration (x 10⁹/mL) in matured Boer bucks fed with the dietary treatments at month 0, month 2, month 4, and month 6 of the feeding trial (±SEM). Note: [a,b]Superscript are significantly different (p<0.05) in the bars. SEM= Standard error of the mean. Group A= Supplemented with 0.18 mg organic Se/kg DM; Group B= Supplemented with 0.36 mg organic Se/kg DM; Group C= No Se supplementation (control). The number of replicates for each treatment was n=6. □ Group A ▨ Group B ■ Group C

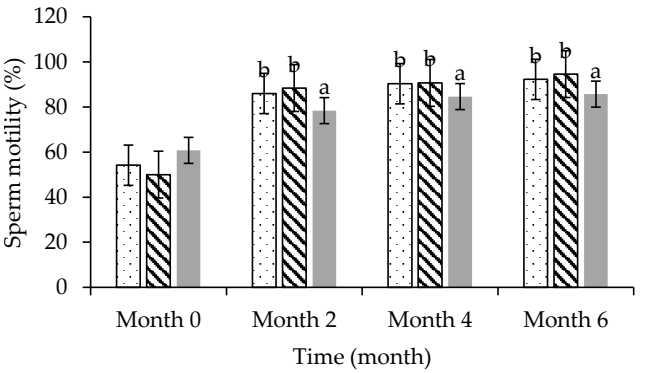

Figure 5. Sperm motility (%) in matured Boer bucks fed with the dietary treatments at month 0, month 2, month 4, and month 6 of the feeding trial (±SEM). Note: [a,b]Superscript are significantly different (p<0.05) in the bars. SEM= Standard error of the mean. Group A= Supplemented with 0.18 mg organic Se/kg DM; Group B= Supplemented with 0.36 mg organic Se/kg DM; Group C= No Se supplementation (control). The number of replicates for each treatment was n=6.
□ Group A ▨ Group B ■ Group C

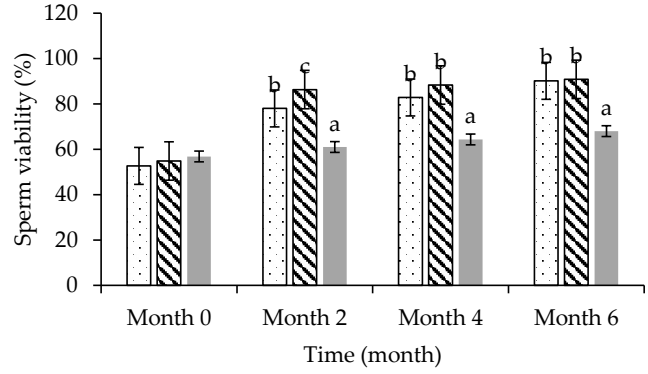

Figure 6. Sperm viability (%) in matured Boer bucks fed with the dietary treatments at month 0, month 2, month 4, and month 6 of the feeding trial (±SEM). Note: [a,b]Superscript are significantly different (p<0.05) in the bars. SEM= Standard error of the mean. Group A= Supplemented with 0.18 mg organic Se/kg DM; Group B= Supplemented with 0.36 mg organic Se/kg DM; Group C= No Se supplementation (control). The number of replicates for each treatment was n=6.
□ Group A ▨ Group B ■ Group C

Table 4. Sperm quality of matured Boer bucks fed with the dietary treatments (±SEM)

	Variables				
	Semen volume (mL)	Sperm concentration (x 10^9/mL)	Sperm motility (%)	Sperm viability (%)	Sperm abnormality (%)
Dietary treatments					
Group A	0.98±0.07[b]	2.21±0.11[b]	89.51±1.15[b]	83.67±1.80[b]	20.11±2.66[b]
Group B	1.04±0.06[b]	2.38±0.19[b]	91.23±1.10[b]	88.50±1.14[c]	13.06±0.88[a]
Group C	0.57±0.05[a]	1.52±0.06[a]	82.90±1.05[a]	64.44±1.47[a]	45.89±3.13[c]
P-value					
Treatment	<0.01	<0.01	<0.01	<0.01	<0.01
Time	0.98	<0.01	<0.01	<0.01	<0.01
Treatment*time	0.84	0.30	0.80	0.55	<0.01

Note: [a,b,c]superscript are significantly different (p<0.05) in the same column; SEM= Standard error of the mean. Group A= Supplemented with 0.18 mg organic Se/kg DM; Group B= Supplemented with 0.36 mg organic Se/kg DM; Group C= No Se supplementation (control). The number of replicates (each parameter) for Se treatments and time, respectively n=18.

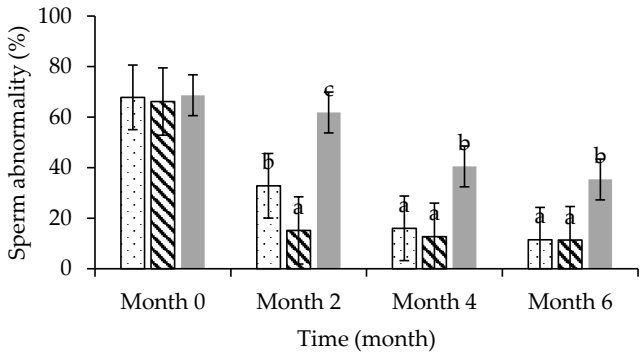

Figure 7. Sperm abnormality (%) in matured Boer bucks fed with the dietary treatments at month 0, month 2, month 4, and month 6 of the feeding trial (±SEM). Note: [a,b]Superscript are significantly different ($p<0.05$) in the bars. SEM= Standard error of the mean. Group A= Supplemented with 0.18 mg organic Se/kg DM; Group B= Supplemented with 0.36 mg organic Se/kg DM; Group C= No Se supplementation (control). The number of replicates for each treatment was n=6.
□ Group A ▨ Group B ■ Group C

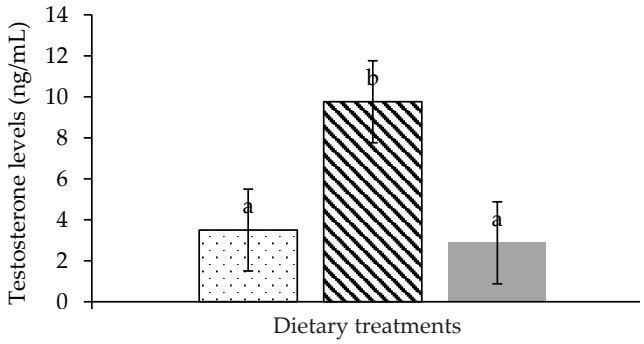

Figure 8. Testosterone levels (ng/mL) in matured Boer bucks fed with the dietary treatments at the end of the feeding trial (±SEM). Note: [a,b]Superscript are significantly different ($p<0.05$) in the bars. SEM= Standard error of the mean. Group A= Supplemented with 0.18 mg organic Se/kg DM; Group B= Supplemented with 0.36 mg organic Se/kg DM; Group C= No Se supplementation (control). The number of replicates for each treatment was n=6.
□ Group A ▨ Group B ■ Group C

mg/kg DM (Marai *et al.*, 2009). These findings seem to be the first result to identify the cause of Se deficiency on low libido in matured Boer bucks, and at the same time to rectify this problem by Se supplementation. These findings are also useful in improving the libido of adult bucks by Se supplementation, even at the maintenance stage.

There was no significant effect of Se supplementation on the scrotal circumference of Boer bucks. However, the scrotal circumference in this study was larger than the recommended to be used in the breeding goats (more than 25 cm) (Tibary *et al.*, 2018). Ghorbani *et al.* (2018) also reported that Se supplementation at 0.30 mg/kg DM did not affect the scrotal circumference in matured rams. In contrast, Lukusa & Lehloenya (2017) reported that Se supplementation at 0.30 mg/kg DM

Table 5. Correlations (r) between scrotal circumference with libido and sperm quality of matured Boer bucks fed with the dietary treatments

Variables	r
Scrotal circumference and libido	-0.37**
Scrotal circumference and semen volume	0.37**
Scrotal circumference and sperm concentration	0.21
Scrotal circumference and sperm motility	0.50**
Scrotal circumference and sperm viability	0.45**
Scrotal circumference and sperm abnormality	-0.41**

Note: ** Significant at ($p<0.01$); r= Value for correlation. The number of replicates for each variable was n= 72.

improved ($p<0.05$) the scrotal circumference in matured Saanen bucks. The antioxidant effect of Se involved in the development and stability of the membrane cells influences the gross and histological morphology of the testis (Ahsan *et al.*, 2014). However, the effect of Se supplementation on the scrotal circumference variations depends on time, age, and breed (Bano *et al.*, 2018). In this case, matured Boer bucks showed less responsiveness to Se supplementation on the scrotal growth. It could also be due to the limited number of animals used (only 18 bucks) and the short study duration (only six months). More animals, preferably 60 bucks and a longer period (for example, 12 months duration), are needed to get better variability and significant results.

Sperm Quality

Initially, all bucks showed low sperm quality, where their semen volume was less than 1.0 mL, sperm concentration was less than 2.5 x 10^9/mL, less than 75% of sperm motility, and more than 15% of sperm abnormality (Yodmingkwan *et al.*, 2016). This finding indicated that deficiency of Se could affect the sperm quality of Boer bucks.

In the present study, the semen volume of Boer bucks was affected ($p<0.05$) by Se supplementation at 0.18 mg/kg DM and 0.36 mg/kg DM. Semen volume in the bucks supplemented with 0.36 mg Se/kg DM (1.04 mL) was the acceptable value mentioned by Yodmingkwan *et al.* (2016) in the standard matured Boer bucks (1.0 mL). A similar finding by Lukusa & Lehloenya (2017) reported that semen volume was affected by Se supplementation at 0.30 mg/kg DM ($p<0.05$) in matured Saanen bucks. Semen volume was also significantly affected ($p<0.05$) by Se supplementation at 0.30 mg/kg DM in Saanen kid bucks (Mojapelo & Lehloenya, 2019). Se supplementation at the levels of 0.50 mg/kg DM, 1.0 mg/kg DM, and 2.0 mg/kg DM in matured Taihang Black bucks also significantly affected ($p<0.05$) the semen volume (Shi *et al.*, 2010). The increased semen volume could be attributed to Se being involved in developing primary and secondary sex glands, resulting in the increased seminal plasma secretion (Lukusa & Lehloenya, 2017; Mojapelo & Lehloenya, 2019). Time did not affect the semen volume. Semen volume could be influenced by other factors such as the method of semen collection, seasons, and environmental temperature (Isnaini *et al.*, 2020; Suyadi, 2012).

Sperm concentration was increased (p<0.05) by Se supplementation at 0.18 mg/kg DM and 0.36 mg/kg DM. This result was in agreement with Lukusa & Lehloenya (2017) and Mojapelo & Lehloenya (2019), who reported that Se supplementation at 0.30 mg/kg DM affected (p<0.05) the sperm concentration in matured Saanen bucks and Saanen kid bucks, respectively. Se supplementation at 0.50 mg/kg DM, 1.0 mg/kg DM, and 2.0 mg/kg DM in matured Taihang Black bucks also significantly affected (p<0.05) the sperm concentration (Shi *et al.,* 2010). The increase in sperm concentration could be linked to the previous studies showing that Se supplementation correlated with Se concentration in the testis. Thus, Se will support and protect sperm cells through an antioxidant agent, glutathione peroxidase (GSH-Px), during the production and maturation of sperm (Bano *et al.,* 2018; Lukusa & Lehloenya, 2017; Mojapelo & Lehloenya, 2019).

Sperm motility is an important factor that influences the fertility of male animals (Hahn *et al.,* 2019). Sperm motility was increased (p<0.05) by Se supplementation at the levels of 0.18 mg/kg DM and 0.36 mg/kg DM. Both Se supplementations had higher sperm motility (89.51% and 91.23%, respectively) than that reported in the standard matured Boer bucks (75%) by Yodmingkwan *et al.* (2016). A similar finding reported that the sperm motility was significantly affected (*p*<0.05) by Se supplementation at 0.30 mg/kg DM in the matured Saanen bucks (Lukusa & Lehloenya, 2017) as well as in Saanen kid bucks (Mojapelo & Lehloenya, 2019). Se supplementation at 0.50 mg/kg DM, 1.0 mg/kg DM, and 2.0 mg/kg DM in matured Taihang Black bucks also significantly affected (p<0.05) the sperm motility (Shi *et al.,* 2010). The increased sperm motility could be attributed to the antioxidant agent (GSH-Px), and ATP metabolism of spermatozoa was found higher in the semen of bucks supplemented with Se. It will reflect the greater metabolism of mitochondrial in spermatozoa and increase sperm motility (Shi *et al.,* 2010). Further study should be conducted to evaluate the effect of Se supplementation in matured Boer bucks on fertility (conception, kidding rate, and pregnancy length in does).

Sperm viability was increased (p<0.05) by Se supplementation at 0.18 mg/kg DM and 0.36 mg/kg DM. Se supplementations had higher sperm viability with 83.67% and 88.50%, respectively than that reported in the elite matured Boer bucks (80.50%) (Suyadi, 2012). Previous studies showed that the sperm viability was significantly affected (p<0.05) by Se supplementation at 0.30 mg/kg DM in the matured Saanen bucks (Lukusa & Lehloenya, 2017) and Saanen kid bucks (Mojapelo & Lehloenya, 2019). Se supplementation at 0.50 mg/kg DM, 1.0 mg/kg DM, and 2.0 mg/kg DM in matured Taihang Black bucks also significantly increased (p<0.05) the sperm viability (Shi *et al.,* 2010). The positive effect of Se on sperm viability could be due to the Se effect via an antioxidant agent (GSH-Px) to protect the formation and maturation of spermatozoa (Ahsan *et al.,* 2014; Mojapelo & Lehloenya, 2019). The sperm quality and fertility generally depend on the maturation of spermatozoa (Bano *et al.,* 2018).

Sperm abnormality was decreased (p<0.05) by Se supplementation at 0.18 mg/kg DM and 0.36 mg/kg DM. Sperm abnormality in the bucks supplemented with 0.36 mg Se/kg DM (13.06%) was less than the sperm abnormality value (15%) mentioned in standard matured Boer bucks (Yodmingkwan *et al.,* 2016). In matured Saanen bucks and Saanen kid bucks, sperm abnormality had significantly decreased (p<0.05) when they were fed with Se supplementation at 0.30 mg/kg DM (Lukusa & Lehloenya, 2017; Mojapelo & Lehloenya, 2019). Se supplementation at 0.50 mg/kg DM, 1.0 mg/kg DM, and 2.0 mg/kg DM in matured Taihang Black bucks also significantly decreased (p<0.05) the sperm abnormality (Shi *et al.,* 2010). The decreased sperm abnormality could be attributed to the role of Se involved in the activity of selenoprotein P during the spermatogenesis process (Bano *et al.,* 2018). Besides, Se protects the lipid component in the sperm acrosome and increases the integrity of the sperm acrosome (Lukusa & Lehloenya, 2017). Interaction between Se treatments and time (month) was found (p<0.05) on the sperm abnormality. It may be due to the organic Se used in this study being more retained in testes than inorganic Se, leading to the continuous effect of Se on the testes (Bano *et al.,* 2018). The continuous effect of Se directly on the interstitial tissue of testes will protect and avoid any disruption during the spermatogenesis process. Thus, the sperm abnormality will be decreased (Ahsan *et al.,* 2014; Ghorbani *et al.,* 2018; Mojapelo & Lehloenya, 2019).

The improvement in sperm quality is also related to the scrotal circumference. In this study, scrotal circumference was related to semen volume (r= 0.37, p<0.01), sperm motility (r= 0.50, p<0.01), sperm viability (r= 0.45, p<0.01) and sperm abnormality (r= -0.41, p<0.01). It may be due to the scrotal circumference in this study being more than 25 cm could develop more germs, and Sertoli cells lead to produce more spermatozoa (Mojapelo & Lehloenya, 2019; Tibary *et al.,* 2018). However, scrotal circumference had no relationship with sperm concentration (r= 0.21, p>0.05). Sperm concentration could be influenced by other factors such as the method of semen collection, seasons, and environmental temperature (Isnaini *et al.,* 2020; Suyadi, 2012).

The quality of sperm is also influenced by testosterone levels. Higher testosterone levels will lead to higher-quality sperm (Armansyah *et al.,* 2018). The quality of sperm is also influenced by season. In a tropical climate, the quality of sperm was increased in the rainy season than in the dry season (Isnaini *et al.,* 2020; Suyadi, 2012). Further study should be conducted to determine the effect of Se supplementation on the dry season to make efficient sperm production throughout the year. The quality of sperm also improved when bucks were fed with adequate nutrition (Abdullah *et al.,* 2015). More importantly, Se supplementation gave more benefit in improving the quality of sperm than the control group (deficient in Se).

Testosterone Levels

The bucks supplemented with 0.36 mg Se/kg DM had high serum testosterone levels (p<0.05) compared

to the bucks supplemented with 0.18 mg Se/kg DM and the control group. The lack of responses in Boer bucks supplemented with 0.18 mg Se/kg DM is understandable as the level of supplemented Se was within the adequacy range of 0.10 mg/kg DM to 0.30 mg/kg DM (NRC, 2007). Mojapelo & Lehloenya (2019) reported that Se supplementation at the level of 0.30 mg/kg DM had a significant effect ($p<0.05$) on serum testosterone levels in Saanen kid bucks. Matured Barbari bucks had a significant increase ($p<0.05$) in serum testosterone levels when they were fed with Se at the level of 0.50 mg/kg DM (Kumar *et al.,* 2013). Se supplementation at the levels of 0.50 mg/kg DM, 2.0 mg/kg DM, and 4.0 mg/kg DM had a significant effect ($p<0.05$) on serum testosterone levels in Taihang Black kid bucks (Shi *et al.,* 2018). Se acts indirectly stimulates the anterior pituitary to release a follicle-stimulation hormone (FSH) and luteinizing hormone (LH) (Ghorbani *et al.,* 2018). When LH increases, it will be secreting a higher testosterone level in the bucks (Lukusa & Lehloenya, 2017).

In a different study, Ghorbani *et al.* (2018) reported that serum testosterone levels were not affected by Se supplementation at the level of 0.30 mg/kg DM in rams. Variations in the results were attributed to the different species and breeds used in those studies. Species and breeds responded differently to testosterone levels (Armansyah *et al.,* 2018). For example, matured Boer bucks in this study had a high testosterone level of 9.76 ng/mL compared to 5.21 ng/mL in matured Barbari bucks when they were supplemented with Se. Boer bucks could be more responsive to Se supplementation than the Barbari bucks, resulting in the increased concentration of male hormones.

CONCLUSION

As expected, Se deficiency affected the reproductive performance of Boer bucks, which includes the reduced libido, quality of sperm, and testosterone levels. Supplementation of Se at a level of 0.18 mg/kg DM has affected the libido and sperm quality. Adequate Se is necessary for sperm cell formation, particularly in the maturation of spermatozoa. Selenium supplementation at a level of 0.36 mg/kg DM affected the reproductive performance of Boer bucks by increasing their libido, quality of sperm, and testosterone levels. These findings revealed that reproductive performance could be improved with Se supplementation at the level of 0.36 mg/kg DM, although it occurred at the adult stage.

CONFLICT OF INTEREST

We certify no conflict of interest with any financial, personal, or other relationships with other people or organizations related to the material discussed in the manuscript.

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