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Effect of selenium on semen quality and fertility of rams

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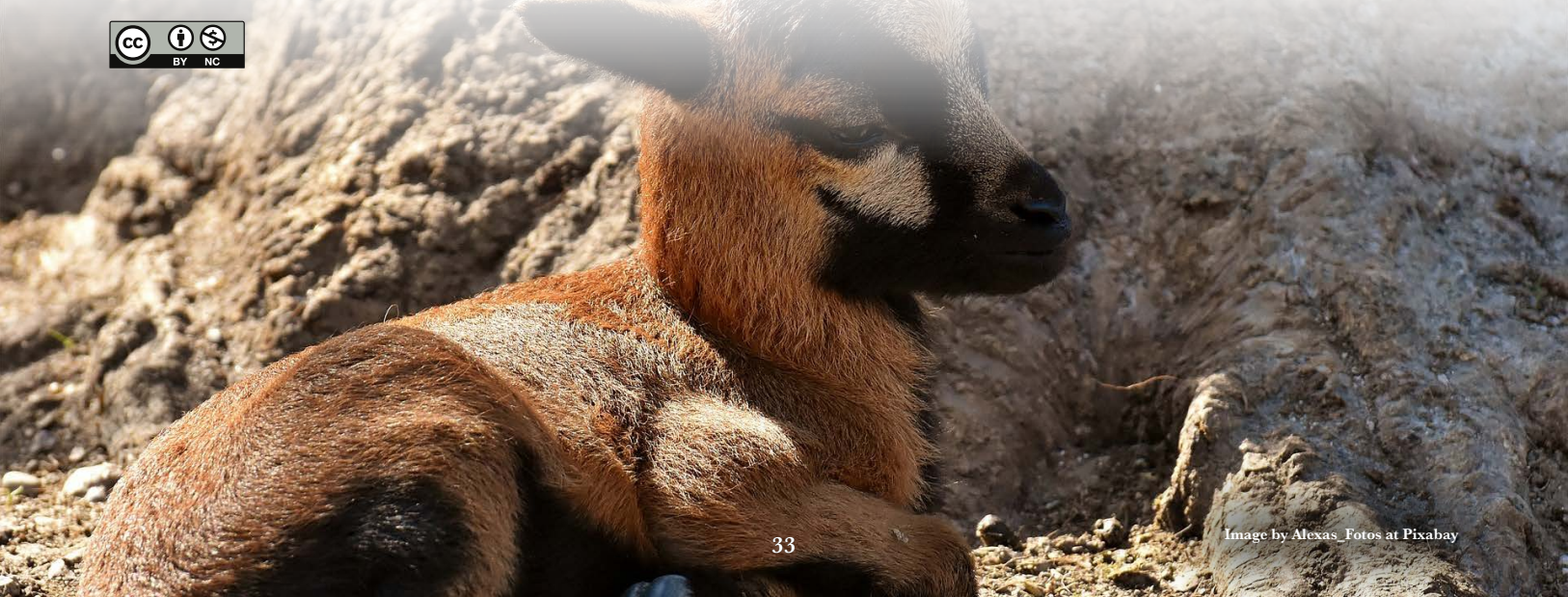
ABSTRACT

Objective: To evaluate the effect of different selenium (Se) doses on semen quality and fertility of rams.
Design/Methodology/Approach: Se was administered subcutaneously each month to nine two-month-old rams (from April 2018 to February 2020). The treatments were: T1=without Se, T2=0.1 mg Se kg^{-1}, and T3=0.3 mg Se kg^{-1}. Semen evaluations were carried out using an electroejaculator; a total of 180 ejaculations were analyzed. The rams with better semen quality (one per treatment) were subjected to a mating. The following variables were evaluated: volume (Vol), masal motility (MM), progressive motility (MP), sperm concentration per mL and ejaculation, acrosome integrity percentage, living spermatozoa, normal spermatozoa, and fertility percentage. The normal distribution variables were evaluated using an analysis of variance (ANOVA) and Tukey's comparison test (Tukey, 0.05); the percentages were raised to the inverse sine in order to perform the appropriate ANOVA. Fertility was analyzed using a χ^2 test.
Results: There was no difference in semen quality and fertility percentage ($p>0.05$). The ejaculation volume showed differences between treatments ($p\leq0.05$).
Study Limitations/Implications: A study including a higher number of rams and a previous fieldwork practice should be carried out, in order to evaluate semen parameters. These evaluations would help to accurately estimate semen quality and fertility, as well as to corroborate the results.
Findings/Conclusions: Se did not improve semen quality and fertility of rams.

Key words: Selenium, ovine, semen, electroejaculator, infertility.



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INTRODUCTION

Selenium (Se) is an essential trace element to keep the physiological balance between animals (Kruzhel *et al.*, 2014). In Mexico, soils and forage do not have enough Se, as a result of the volcanic origin of the soils and erosion. Other elements, such as sulphur and mercury, hinders its absorption by ruminants (29-35%). Additionally, ruminal bacteria capture Se for their own metabolism (Ramírez-Bribiesca *et al.*, 2001). Therefore, the Se requirements of animals must be met in regions with deficient soils and forages (Saha *et al.*, 2016). Currently, there are several options to provide Se to animals. It can be provided through organic or inorganic mineral salts, which can be included in the diet, water, mineral blocks, solutions for injections, or sustained release methods, such as ruminal bolus (Díaz-Sánchez *et al.*, 2019).

On the one hand, low Se concentrations are related to productive and reproductive problems in small ruminants (Mahmoud *et al.*, 2013), as a result of their peroxide plasma levels, which quickly increase and damage capillary endothelium, red blood cells, seminal plasma, and muscle fibers (Monroy, 2017); these concentrations also cause nutritional muscular dystrophy (white muscle disease), lameness, and growth reduction (Tapia, 2015). On the other hand, a fertility reduction has also been recorded in rams that have a low semen quality (low sperm counting and an increase in sperm abnormalities) (Beckett and Arthur, 2005).

The inclusion of Se in diets or its parenting applications has improved weight gain in lambs, fertility in ewes, and immune responses in sheep (Carbajal *et al.*, 2013). Se also improved the antioxidative state, increased the testosterone and triiodothyronine (T3) levels in seminal plasma and blood serum, and protected spermatozoa from oxidative damage in bucklings, which is an important factor in the production of good quality semen (Kumar et al., 2013). Therefore, the objective of this study was to evaluate the effect of different doses of Se in semen quality and fertility of rams.

MATERIALS AND METHODS

Study area

The research was carried out from April 2019 to February 2020 in the Lomas de San Rafael production unit, in Suchiapa, Chiapas, Mexico. The production unit is located at 16° 40' 0" N and 93° 04' 53" W, at 695 masl. The climate is subhumid warm with summer rains; the mean annual temperature and total annual precipitation ranges from 20 to 28 °C and from 800 to 1200 mm, respectively (INEGI, 2017).

Experimental animals

Nine two-month-old, recently weaned Blackbelly rams were used in the experiment. The selection of animals and their distribution per treatments were completely random. Animals who weighed less than 12 kg and were one- or three-month-old were rejected. The rams were placed in a stable and received a corn-soy based diet, with 13.44% of raw protein, 4.35 kcal, and free access to water. Treatments were: T1, without Se (control); T2, 0.1 mg Se kg^{-1}; and T3, 0.3 mg Se kg^{-1}. Each treatment had three repetitions (three rams). Se, was provided subcutaneously each month, from April 2018 to February 2020;

a commercial brand —which included 10.95 mg sodium selenite, equivalent to 5 mg of selenium and vitamin E— was used.

Seminal evaluation

In order to carry out the seminal analysis, two ejaculations from each ram were collected every fortnight, until the end of the study, and the macroscopic and microscopic characteristics of a total of 180 ejaculations were evaluated. The semen samples were obtained using a Bailey electroejaculator. In order to obtain the samples, each ram was placed in an ulna lateral position; subsequently, the prepuce area was cleaned, using anti-bacterial wipes. The electroejaculator was then introduced anally and operated in 3-5 second cycles, with a 3 second rest. The ejaculation was gathered using a cone attached to one of the ends of a collecting tube; a cover was used to protect the collecting tube from sunlight. This technique is time-efficient and does not require to train rams in order to collect semen (Arieta *et al.*, 2014).

The following seminal variables were evaluated: 1) Ejaculation volume (Vol.): it was determined using a measuring cylinder, with gradations of 0.1 mL-15 mL units (Carrillo-González and Hernández, 2016). The reading was carried out by direct observation of the collecting tube, taking advantage of its transparency. 2) Masal motility (MM): it was carried out at the time when each sample was collected. A sample of each ejaculation was placed in a microscope slide and was observed in a microscope with a 10X objective. Subsequently, a 0-5 scale value (0=minimum; 5=maximum) was provided to each sample; the values given to the samples depended on the movement vitality of the waves and the individual observation of the spermatozoa (Lozano *et al.*, 2016). 3) Progressive motility (MP): it was carried out in a 1:100 dilution (9.9 mL saline solution and 0.1 mL of each ejaculation); it was diluted and a small drop was observed in the microscope with a 10X objective. Subsequently, a value was given depending on the progressive movement of the spermatozoa (Benítez-González *et al.*, 2018). 4) mL sperm concentration (Conc. Esp. mL): A Neubauer chamber and a 1:200 dilution (1 mL saline solution and 1 mL diluted semen, previously prepared for the MP evaluation) were used (Carrillo-González and Hernández, 2016). The Neubauer chamber carrying the semen sample was observed in a microscope with a 40X objective. Subsequently, the heads of the spermatozoa located inside the five boxes of the two compartments were counted. Then, the average of the spermatozoa counted in the two compartments was multiplied by 10^7. 5) Sperm concentration per ejaculation (Conc. Esp. Ey.): the Conc. Esp. mL was multiplied by the volume of each ejaculation. 6) Complete acrosome percentage: a smear was carried out, using a 1:100 dilution, previously prepared with the Spermac Stain®. A 100X objective and immersion oil were used to observe the spermatozoa with the blue acrosome. In order to obtain the percentage value, 200 spermatozoa were counted and classified according to complete and damaged acrosome (Mancheno and Díaz, 2018). 7) Living spermatozoa percentage: it was obtained smearing an eosin-nigrosine stain, together with the diluted semen of each sample. Living and dead spermatozoa were counted in a microscope with a 40X objective. Living spermatozoa had a light color throughout their structure, while dead spermatozoa had a consistent dark color. A total of 100 spermatozoa were evaluated (Malejane *et al.*, 2014).

8) Normal spermatozoa percentage: a 1:100 dilution (prepared beforehand) was smeared using a Spermac Stain®. They were observed with a 100X objective and immersion oil. In order to evaluate normal spermatozoa, 200 spermatozoa were counted. They were classified as normal or with primary and secondary abnormalities.

Fertility evaluation

One ram per group was selected and those chosen had a good semen quality throughout the study; additionally, a mating was carried out for 60 days in a stable; for this purpose, 20 adult ewes per ram were selected. The pregnancy was established by means of a transrectal ultrasound; the fertility percentage was determined per each treatment.

Statistical analysis

The semen characteristics were analyzed using the generalized linear model procedure (PROC GLM), through a completely random design; the characteristics evaluated (%) were raised to the inverse sine before the analysis took place. The Tukey Test (0.05) was used to compare the means of the treatments. Fertility was evaluated using the χ^2 test, in 3×2 contingency tables. All the statistical analysis were carried out using the SAS software (SAS, 2016).

RESULTS AND DISCUSSION

Semen quality analysis

The volume of the ejaculations was higher in rams treated with 0.3 mg Se kg^{-1} than in the rest of the treatments. The averages recorded in this study were similar to those obtained by Mahmoud *et al.* (2009), who found statistical differences in the volume of the ejaculations of rams treated with Se (0.97 mL) and without Se (0.84 mL). The increase in the volume of ejaculations is related to the action of Se and can be observed in the development of the primary and secondary sex glands, the spermatogenesis, and the prostate function, which increases the seminal plasma secretion (Kolodziej and Jacyno, 2005). The volume of the ejaculations found in this study differ from the results obtained

Table 1. Seminal variables of rams treated with different doses of selenium (Se).

Variables	Se (mg kg^{-1})		
	0.0	0.1	0.3
Volume (mL)	0.68±0.1b	0.79±0.1ab	0.91±0.12a
Mass motility (1 a 5)	2.51±0.3a	2.56±0.3a	2.86±0.4
Progressive motility (%)	60.18±12a	51.35±12a	62.88±12
Concentration mL^{-1} ($\times 10^7$)	137.56±59a	120.44±58a	132.5±52
Ejaculate concentration^{-1} ($\times 10^7$)	93.54±35a	95.15±35a	120.58±40
Whole acrosome (%)	86.53±12a	87.63±10a	87.59±13
Live sperm (%)	81.26±10a	80.90±10a	80.90±10
Normal spermatozoa (%)	84.2±10a	84.73±10a	84.61±10

Means ± EE with different letter in the same row are different ($p \leq 0.05$).

by Carrillo-Nieto *et al.* (2018), who used ruminal bolus with a Se (0.87 mL) supplement and did not record differences ($p>0.05$) regarding the control group (0.91 mL).

There were no differences regarding masal motility between treatments; these results match the findings of Duvos *et al.* (2017), who subcutaneously provided a Se supplement (2.54) to the animals, but did not record differences with the control group (2.50). These findings can be the result of a difference in sperm motility. Se is one of the co-factors of the glutathione peroxidase enzyme (GSH-Px), a powerful antioxidant that protects the sperm cells from the damage caused by reactive oxygen species (ROS; Tareq *et al.*, 2010).

Progressive motility was similar between treatments ($p>0.05$). The means recorded in this research are similar to those found by Baiomy *et al.* (2009), who did not record statistical differences in rams with 0.2 mg kg^{-1} and 0.5 mg kg^{-1} Se treatments, obtaining an average of 82.5 and 86.67% progressive motility, respectively.

Sperm concentration per mL was similar between treatments ($p>0.05$). Regarding these results, Baiomy *et al.* (2009) and other authors have reported that adding inorganic Se to ram and bull diets does not improve semen quality. Additionally, Lovecamp *et al.* (2013) found that Se supplementation did not change the quality and quantity of fresh ejaculations in pigs. Therefore, the results of this research match those obtained by Carrillo-Nieto *et al.* (2018), who used ruminal bolus supplemented with Se (185 spermatozoa mL^{-1}). They did not record statistical differences regarding control (182 spermatozoa mL^{-1}). Finally, Duvos *et al.* (2017) reported 259 spermatozoa mL^{-1} in animals that had received a Se supplement applied subcutaneously. Control had statistically similar results (262 spermatozoa mL^{-1}).

Se, did not increase the sperm concentration per ejaculation ($p>0.05$). The amount of Se in the supplement may not be enough to produce selenoproteins which help the Sertoli cells proliferation in the developing testicles. In its turn, Sertoli cells proliferation leads to an increase in the number of spermatozoa, which increases sperm concentration (Ahsan *et al.*, 2014). The results of this research differ from the findings of Mahmoud *et al.* (2013), who recorded statistical differences with Ossimi sheep that received Se supplement through an intramuscular injection; the results of the treatments with Se and without Se were 106 and 314 ejaculated spermatozoa^{-1}, respectively.

There was no difference between treatments regarding the living spermatozoa percentage ($p>0.05$). These results differ from those recorded by Carrillo-Nieto *et al.* (2018), who reported 92.18% and 93.94% results in rams fed with ruminal bolus, with and without a Se supplement, respectively.

The normal sperm percentage was similar between treatments ($p>0.05$). These results do not match those obtained by Marín-Guzmán *et al.* (2000), who proved that sperm morphology is related to Se supplementing.

Fertility analysis

Cerri *et al.* (2009) proved that both the Se deficiency and the excess of reactive oxygen species (oxidants) has a negative impact on fertility. Se treatments in this research achieved better quality values (Table 2), as a result of the increased number of spermatozoa per ejaculation and the oocyte integrity of the ewes (Das *et al.*, 2006); however, no differences were recorded ($p>0.05$).

Table 2. Ovine fertility with different Se treatments.

Se (mg kg^{-1})	Females in pairing		
	Pregnant females	Non-pregnant females	Fertility percentage
0.0	19	1	95a
0.1	20	0	100a
0.3	20	0	100a

Means with the same letter in the column are no different ($p>0.05$).

CONCLUSIONS

The subcutaneous application of Se did not improve the development, semen quality, and fertility of the rams; however, a 0.3 mg kg^{-1} of Se dose significatively increased the volume of the ejaculation.

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