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Evaluation of biochemical and electrolytic components of semen from ram supplemented with different concentrations of selenium and its correlation with sperm quality

[*Avaliação de componentes bioquímicos e eletrolíticos do sêmen de ovinos suplementados com diferentes concentrações de selênio e sua correlação com a qualidade espermática*]

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ABSTRACT

The aim of the study was to evaluate whether supplementation with different levels of selenium (Se) can change the biochemical and electrolytic components of semen, causing an improvement in seminal quality in rams. Thirty animals were kept in an intensive pen system, fed with hay and commercial ration, allocated into five groups (six animals/pen) and supplemented with a mineral mixture containing 0mg (G1), 5mg (G2), 10mg (G3), 15mg (G4) and 20mg (G5) of Se/kg. Each group received a different treatment every 56 days and treatments were rotated between groups following a dynamic sequence. Semen samples were collected by electroejaculation after the end of each treatment to evaluate the levels of fructose, citric acid, potassium (K), sodium (Na), calcium (Ca), Se, zinc (Zn), manganese (Mn), sulfur (S) and lead (Pb). The statistical design was a 5x5 Latin square. The different levels of Se supplementation evaluated maintained the concentrations of electrolytes and minerals in the semen at the required levels and did not change the sperm quality, concluding that higher intakes of Se do not cause antagonistic effects on the absorption and subsequent action of other essential minerals supplied to the animals and still maintains electrolyte balance.

Key words: ovine, ejaculate, supplementation, micromineral

RESUMO

O objetivo do estudo foi avaliar se a suplementação com diferentes níveis de selênio (Se) pode alterar os componentes bioquímicos e eletrolíticos do sêmen, ocasionando, por conseguinte, uma melhoria na qualidade seminal em carneiros. Foram utilizados 30 animais, mantidos em sistema intensivo de baias, alimentados com feno e ração comercial, sendo alocados em cinco grupos (seis animais/baia) e suplementados com uma mistura mineral contendo 0mg (G1), 5mg (G2), 10mg (G3), 15mg (G4) e 20mg (G5) de Se/kg. Cada grupo recebeu um tratamento diferente a cada 56 dias, e foi realizado um rodízio de tratamentos entre os grupos seguindo uma sequência dinâmica. Amostras de sêmen foram colhidas por eletroejaculação após o fim de cada tratamento, a fim de se avaliarem os níveis de frutose, ácido cítrico, potássio (K), sódio (Na), cálcio (Ca), Se, zinco (Zn), manganês (Mn), enxofre (S) e chumbo (Pb). O desenho estatístico foi um quadrado latino 5x5. Os diferentes níveis de suplementação de Se avaliados mantiveram as concentrações de eletrólitos e de minerais do sêmen nos níveis exigidos e não modificaram a qualidade espermática, concluindo-se que maiores ingestões de Se não causam efeitos antagônicos na absorção e subsequente ação de outros minerais essenciais fornecidos aos animais e ainda mantêm o equilíbrio eletrolítico.

Palavras-chave: ovino, ejaculado, suplementação, micromineral

INTRODUCTION

Sheep husbandry in Brazil is an important livestock activity with a herd of approximately 19.7 million animals (Pesquisa…, 2019). Therefore, to optimize the activity it is necessary to increase productivity and profitability (Garcia, 2003; Lobato *et al*., 2013).

Reproductive efficiency becomes indispensable for a more profitable system (Mori *et al*., 2006), as the production becomes technified, it allows an increase of reproductive and productive rates,

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as well as the genetic improvement in the herd (Souza *et al*., 2008).

Reproductive physiology on sperm production capacity and its quality is essential knowledge for the high performance of male breeders (Costa *et al*., 2015). Therefore, nutritional management is one of the most important factors in a production system (Digby *et al*., 2011), it can impact semen quality characteristics in males and consequently the development of the reproductive activity (Bindari *et al*., 2013).

Studies were performed to evaluate the mineral influence of the micronutrients on reproductive parameters and if the Se decreases the percentage of sperm defects (Piagentini *et al*., 2017). Se is an important mineral related to ruminant reproduction, and its benefits include spermatogeneses improvement and testicular development (Mahmoud *et al*., 2013). Therefore, the present study aimed to determine the biochemical and mineral components in the semen of rams, supplemented with Se, to evaluate the influence of this micromineral on sperm quality and the levels of other minerals and electrolytes present. Our hypothesis is that high concentrations of Se can alter the absorption of other minerals and alter the electrolyte balance in the seminal plasma of ram, leading to a decrease in seminal quality.

MATERIAL AND METHODS

All experimental procedures were reviewed and approved by the Institutional Ethics Committee on Animal Use (CEUA) Animal Care and Use Committee (Protocol code 180/2014) of São Paulo State University (UNESP), Botucatu, São Paulo, Brazil.The experiment was performed at the city of Lara, São Paulo, Brazil, subtropical climate, an average temperature of 26ºC, a latitude of 22º52'15" south, and a longitude of 49º09'46" west at an altitude of 648 meters.

Thirty reproductive mature rams were kept in an intensive stall system and fed with hay and commercial food. The experimental designer of this project was based on the 5x5 latin square design. The animals were randomly allocated to five experimental groups (six animals/ group/stall), five treatments and five repetitions or experimental periods. They were supplemented with a mineral mixture containing the following quantities of Se/kg 0mg, 5mg, 10mg, 15mg, and 20mg. Thus, the experimental groups were control group (CG, 0mg), group 1 (G1, 5mg), group 2 (G2, 10mg), group 3 (G3, 15mg), group 4 (G4, 20mg). The stalls during the experiment period measured 5 meters long and 3 meters wide. The animals had access to water *ad libitum* and to feeders where the supplemented mixture was provided.

A preliminary trial was performed to evaluate the average daily intake of the mineral mixture provided to the animals. The Se was added to this mixture to ensure that the daily amounts of this element were consumed, according to the species reference values.

The CG was supplemented with a mineral mixture without any presence of Se. The hay and commercial food were previously analyzed, before the experiment period, to assure this condition. On day 0 (D0) of the experiment, all the animals were weighed and had a semen sample collected. The first 14 days were considered an adaptation period and adjustment of mineral mixture intake by rams (time to eliminate the residual effect of feed supplied in the previous period). After 14 days, each group received a different treatment every 56 days, with a rotation among groups, always following a dynamic sequence. Between the rotations of the experimental groups, the animals again went through a consumption adjustment period of 14 days without receiving any type of supplementation to eliminate any residues from the previous treatment. Thus, all five groups consumed all mixtures during five experimental periods of 56 days.

The supplied mineral supplement (Ovisal® - Fanton Animal Nutrition) had the following composition per kilogram of product: Ca 89.00g, phosphorus 45.00g, S 1.98g, Mn 2.50g, zinc 2.160.00mg, Mn 300.00mg, cobalt 120.00mg, iodine 8.00mg, Na 97.50g, fluorine (maximum) 450.00mg, the solubility solubility of P205 in citric acid (minimum) 95.00 and, following the manufacturer's recommendation, it was provided the amount of 30g/animal/day. This supplement was added in different concentrations (5mg, 10mg, 15mg, and 20mg/kg of Se) in feeders inside the stalls.

After the end of each treatment period, semen samples were collected by electroejaculation, following to The Brazilian College of Reproduction Animal recommendations (Manual…, 1998).

Seminal quality analysis: Semen samples were collected by electroejaculation after the end of each treatment period. The semen analysis (volume, turbulence, motility, vigor, and sperm concentration) was performed, as recommended by the Brazilian College of Animal Reproduction (CBRA, 1998).

Biochemical analysis of semen Determination of fructose concentration in semen: An aliquot of fresh semen (0,2mL) was diluted with 1 mL of 0.5 N sodium hydroxide and 1 mL of Zn sulfate. After this procedure, samples were put into micro tubes and stored at -20°C for further analysis of fructose concentration levels through using the technique proposed by Pereira and Janini (2001).

Evaluation of plasma citric acid levels in semen: An aliquot of fresh semen (0.2mL) was diluted with 1.8mL of 10% trichloroacetic acid. The samples were stored at -20°C for subsequent analysis of the citric acid concentration described by Saffran and Densted (1948).

Seminal quantification of K, Na and Ca: The concentrations of K, Na, and Ca in the semen were determined by a selective ion electrode (ISE) technique. An aliquot of fresh semen was frozen and subsequently analyzed in the ISE, a sensor that converts the activity of an ion in solution into an electrical potential (Levy, 1981; Kirsztajn, 2010).

Measurement of S, Zn, Mn, S and Pb parameters in semen: The Zn, Mn, and S levels in the semen were determined by the technique of Flame Atomic Absorption Spectroscopy (FAAS) technique and Se and Pb levels were determined by Graphite Furnace Atomic Absorption Spectroscopy (GFAAS). In both methods, an aliquot of fresh semen (0.2mL) was diluted in 0.5% bidistilled nitric acid at a 1:10 ratio and stored at -20°C until analysis, according to described by Athanasopoulos (1994) and Taylor *et al*. (2005).

Statistical analysis: The experimental design of the present study was elaborated as a 5x5 Latin Square, meaning five treatments and five experimental periods or repetitions. The collected data were analyzed using the GLM process as described in SAS (1999). The differences among means were verified with the Tukey test at 5% probability.

RESULTS

The seminal analysis (volume, turbulence, motility, vigor, and concentration) showed no statistical difference among the groups (Table 1).

The concentrations values of fructose, citric acid, K, Na and Ca, are described in Table 2.

Regarding the mineral composition in the ram's semen there was no difference among the concentrations of Se, Zn, Mn, S and Pb (Table 3).

Table 1. The mean values and standard error of volume, turbulence, motility, vigor, and sperm concentration according to the treatment groups

Group	Volume (mL)	Turbulence (1-5)	Motility (%)	Vigor (1-5)	Concentration ($x10^6$)
G1	1.13±0.10	4.67±0.10	87.06±1.17	4.6±0.09	1.30±0.17
G2	1.33±0.09	4.48±0.11	85.23±1.20	4.52±0.10	1.87±0.16
G3	1.27±0.10	4.43±0.10	83.87±1.16	4.49±0.09	1.50±0.16
G4	1.26±0.09	4.49±0.11	84.84±1.17	4.49±0.10	1.28±0.15
G5	1.21±0.10	4.80±0.10	88.14±1.16	4.81±0.09	1.40±0.16

Table 2. Mean ± standard error of fructose and citric acid, Na, P and Ca according to the experimental groups in ram semen in the Control Group (CG; 0 mg Se/kg), Group 1 (G1; 5mg Se/kg); Group 2 (G2; 10mg Se/kg); Group 3 (G3, 15mg Se/kg) and Group 4 (G4, 20mg Se/kg)

Group	Fructose (mg/dL)	Citric Acid (mg/dL)	P (mmol/L)	Na (mmol/L)	Ca (mmol/L)
GC	33.15±2,29	6.09±0,84	9.30±0,85	135.93±1,56	0.52±0,021
G1	25.85±2,34	6.26±0,85	10.83±0,91	136.98±1,68	0.52±0,020
G2	30.15±2,30	5.49±0,83	10.90±0,89	133.46±1,64	0.51±0,021
G3	32.90±2,22	5.94±0,84	10.28±0,87	134.22±1,59	0.49±0,022
G4	30.04±2,35	6.17±0,84	9.42±0,87	135.77±1,59	0.50±0,020

Table 3. Mean ± standard error of Se, Zn, Mn, S and Pb according to the experimental groups in ram semen in Control Group (CG; 0mg Se/kg), Group 1 (G1; 5mg Se/kg); Group 2 (G2; 10mg Se/kg); Group 3 (G3, 15mg Se/kg) and Group 4 (G4, 20mg Se/kg)

Group	Se (µg/dL)	Zn (mg/L)	Mn (mg/L)	S (mg/L)	Pb (mg/L)
GC	0.036±0,004	0.32±0,018	0.36±0,04	17.91±1,29	$1x10^{-3}$±0
G1	0.035±0,005	0.32±0,019	0.28±0,03	13.98±1,25	$1x10^{-3}$±0
G2	0.038±0,004	0.27±0,019	0.39±0,03	18.12±1,47	$1x10^{-3}$±0
G3	0.037±0,004	0.31±0,020	0.32±0,03	14.56±1,33	$1x10^{-3}$±0
G4	0.038±0,004	0.31±0,018	0.36±0,04	18.03±1,95	$1x10^{-3}$±0

DISCUSSION

In general, minerals perform essential functions throughout the body, acting in a beneficial way in the endocrine, reproductive, and immune systems (Piagentini *et al*., 2017). Furthermore, they participate in cellular electrolyte balance, regulating enzymes and membrane proteins responsible for cell transport and metabolism (Smith and Akinbamijo, 2000). Among these minerals, Se stands out, which is closely correlated with the improvement of reproductive functions such as spermatogenesis and fertility in males (Boitani, Puglisi, 2009).

Our study evaluated the effects of increased Se intake on electrolytes and minerals. The concentrations of fructose, citric acid, Na, K and Ca demonstrated a similar seminal biochemical pattern among all the evaluated groups proved supplementation with Se did not alter the concentration levels of these elements in the seminal plasma of rams.

The results of fructose concentrations showed quantitative information on sugars present in the seminal plasma, with an average variation of 25.85 to 33.15mg/dL, among the different treatment groups. These results are similar to those described by Franco (2010), in which the average annual concentration of fructose was 23.40 ± 4.80mg/dL, with a variation of 16.20 to 42.80mg/dL, in the seminal plasma of rams. This information is crucial because the average concentration of fructose is related to fertility due to the energy production by the spermatozoa (Tutida *et al*., 1999).

The dietary levels of Se also did not alter interfere in the citric acid concentration of the seminal plasma of rams, promoting an average variation of 5.49 to 6.26mg/dL among the different treatment groups. The concentrations of citric acid in the seminal plasma of rams according to Aguiar *et al*. (2013), were 502.6 ± 51mg/dL in the dry season and 660.1 ± 39.4mg/dL in the rainy season, and these values were higher than those observed in our study. These differences in the seminal plasma biochemistry may be due to the semen collection method. In our study, the semen collection was by electroejaculation, and in Aguiar *et al*. (2013), it was by artificial vaginal. The sperm activity is dependent on the ionic environment, and seminal plasma has an ionic composition that changes in individuals and species (Kareskoski and Katila, 2008). In the present study, the supplementation with Se did not alter the K, Na, and Ca concentrations on the rams' seminal plasma in all the different concentrations.

The K is correlated with the concentration and percentage of live sperm present in the ejaculate, and Na has a role in maintaining the osmotic pressure (Abdel-Rahman *et al*., 2000). The mean K in the different treatment groups was 9.30 to 10.90mmol/L, while the Na concentration was 133.46 to 136.98mmol/L (Princewill *et al*., 2015).

Similar to the K and Na, Ca is a regulator of sperm physiology due to its involvement with the motility kinetics and triggering the acrosome reaction (Kumar *et al*., 2011). Ca participates in regulating of adenyl-cyclase and the ionic permeability of the sperm membrane, allowing the fertilization process (Jaiswal, Conti, 2003). The average variation in our study was 0.49 to 0.52mmol/L in the groups, demonstrating that Se supplementation did not change the concentrations of this macromineral. Such as Ca, the Zn concentration was similar in the groups (0.27 to 0.32mg / L). Zn has antioxidant activity, and it plays a role in sperm motility. It is also considered an antimicrobial factor for gram-negative and gram-positive bacteria (Barrier-Battut *et al*., 2002). However, no changes were observed after Se supplementation.

The Mn is involved in the free radical formation, which causes a high oxidative action and may promote damage to the sperm membrane and DNA (Reis *et al*., 2014). The average variation of Mn was from 0.28 to 0.39mg/L, and the Se supplementation did not change the concentrations of this mineral.

Moreover, to identify the presence of heavy metals, the Pb dosage was performed, however, these values were below the detection limits, which demonstrate exposure to Pb lower than the reference range.

The addition of Se in ram feed can be a recommended practice considering its beneficial effects during spermatogenesis given its antioxidant and sperm protection functions during the maturation period (Ortunho *et al*., 2016; Piagentini *et al*., 2017). Beneficial actions were observed in semen from ram supplemented with different concentrations of Se, where this mineral promoted an improvement in semen quality, decreasing the percentage of sperm defects, and reducing damage to sperm DNA Piagentini *et al*., 2017; Moya *et al*., 2021). In our study, the different levels of Se supplementation evaluated maintained the concentrations of electrolytes and minerals in the semen of ram at their required levels, to conclude that higher Se intakes do not cause antagonistic effects on the absorption and subsequent action of others essential minerals supplied to animals and still maintains the electrolyte balance.

Thus, due to the beneficial action caused by higher Se ingestion by ram, which showed improvements in their sperm characteristics, this mineral can be added to food supplementation with greater safety and can also help to maintain seminal quality without harming the absorption of other minerals and maintain the electrolyte balance of the semen. We believe that the data obtained in our study provided information with important implications in the animal reproduction field. However, more studies must be done to better detail the bioavailability of the analyzed minerals.

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