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Supplementation of two sources of selenium at different levels in diet of boars and this effect on the seminal quality

Suplementação de duas fontes de selênio em diferentes níveis na dieta de cachaços e seu efeito sobre a qualidade seminal

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Abstract
The spermatic membrane is rich in polyunsaturated fatty acids, which makes it sensitive to the action of reative species of oxygen, which can damage the seminal quality of the scraps. The purpose of this study was to evaluate the effect of the supplementation of two selenium sources at different doses. Third five scraps were allocated in four groups: (INOR30) 0.30 ppm sodium selenite; (COMP30) 0.30 ppm selenium metal-amino acid; (MIXED15+15) 0.15 ppm sodium selenite + 0.15 ppm selenium metal-amino acid and (COMP15) 0.15 ppm selenium metal-amino acid. The ejaculates of the scraps were evaluated over 22 weeks, resulting in 210 samples evaluated for volume, motility, pH, presence of agglutination and morphological changes, and 140 samples for spermatic concentration. The data was analyzed with repeated measures in time in a mixed model with type of selenium supplementation, periods of evaluation (one period of two weeks + five periods of four weeks) and their interactions as fixed effects, and animal and the worker that collected the ejaculates as random effects. Results showed no difference in selenium supplementation with the sources and doses used. In this way, it was possible to verify that the metal amino acid of selenium at the dose of 0.15 ppm promotes the same effect as the diets formulated with 0.30 ppm of sodium selenite.
Keywords: Antioxidant; Boar; Spermatozoid; Reproduction

Resumo
A membrana espermática é rica em ácidos graxos poliinsaturados, o que a torna sensível à ação de espécies reativas de oxigênio, que podem prejudicar a qualidade seminal dos cachaços. O objetivo do presente estudo foi avaliar o efeito da suplementação de duas fontes de selênio em diferentes doses. Trinta e cinco cachaços foram distribuídos em quatro grupos: (INOR30) 0,30 ppm de selenito de sódio; (COMP30) 0,30 ppm de metal-aminoácido de selênio; (MISTO15+15) 0,15 ppm de selenito de sódio + 0,15 ppm de metal-aminoácido de selênio e (COMP15) 0,15 ppm de metal-aminoácido de selênio. Os ejaculados dos cachaços foram avaliados durante 22 semanas, resultando em 210 amostras avaliadas para volume, motilidade, pH, presença de aglutinação e alterações morfológicas, e 140 amostras para concentração espermática. Os dados foram analisados com medidas repetidas no tempo em modelo misto, em que o tipo de suplementação de selênio, os períodos de avaliação (um período de duas semanas + cinco períodos de quatro semanas) e suas interações foram os efeitos fixos, e o animal e o funcionário que coletou os ejaculados foram os efeitos aleatórios. Os resultados obtidos demonstraram não haver diferença na suplementação de selênio com as fontes e doses utilizadas. Com isso, foi possível verificar que o metal-aminoácido de selênio na dose de 0,15 ppm promove o mesmo efeito das dietas formuladas com 0,30 ppm de selenito de sódio.
Palavras-chave: Antioxidante; Reprodutor suíno; Espermatozoide; Reprodução

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Introduction

The membrane that constitutes the swine sperm is rich in polyunsaturated fatty acids, which is important to maintain sperm fluidity and flexibility[1]. On the other hand, this lipid membrane makes the sperm cell sensitive to oxidative damage caused by reactive oxygen species (ROS)[2,3]. Increased studies have associated high levels of ROS with infertility problems in men[4,5].

Selenium (Se) is a trace mineral in animal feed that is part of the glutathione peroxidase (GPx) enzyme, enhancing its antioxidant activity[6]. GPx is part of the membrane defense system, protecting the integrity of the sperm cell from oxidative damage[3]. Furthermore, Se is also involved in the development of the midpiece and Sertoli cells[7], influencing normal testicular function, sperm cell structure, and sperm motility[8].

The most common form of Se supplementation in swine nutrition is with sodium selenite, but its use is lower since in its oxidized form it can bind to other elements, altering its absorption[9], which consequently increases the excretion of this mineral, mainly through urine[10]. Inorganic minerals, after being ingested, undergo decomposition in the intestine before being absorbed, forming free metal ions, which are very reactive and may affect their bioavailability[9].

To improve the absorption of this element and minimize the environmental impact, Se complexed with amino acids of high biological value is used. These metal-amino acids normally use the absorption pathways of the organic molecule to which they are linked, preventing physical-chemical factors from interfering with their absorption, presenting high bioavailability, and is more readily absorbed[10]. With this, it becomes possible to reduce the necessary dose of the mineral in the diet of swine breeders.

Therefore, the study aimed to evaluate different levels of selenium inclusion in the diet and to compare the effect of selenium metal amino acid with sodium selenite on the semen quality of boars.

Material and methods

This study was approved by the Ethics Committee on the Use of Animals of the Palotina Sector of the Federal University of Paraná (CEUA/UFPR-Palotina) under protocol number 40/2016.

Animals

The experiment was carried out in a Semen Production Center (SPC), located in the western region of Paraná. The boars were housed in individual concrete stalls measuring 2.0 m × 2.5 m, equipped with a masonry feeder and a pacifier-type drinker. The boars' age was 1.73 ± 0.93 years (mean ± standard deviation) when they were selected. These animals came from genetic improvement companies and had already undergone training on the dummy, being able to participate in the experiment.

The SPC could house 50 males. During the study period, 40 breeders fit the experimental profile, but five of them had to be excluded from the study during the period of adaptation to the diet, which lasted two months.

Diet and boar distribution

The diet fed to boars was formulated based on the nutritional requirements of males in reproductive activity[11], being isoproteic and isoenergetic, differing only in terms of source and level of inclusion of the micromineral Se (Table 1). The boars were treated twice a day, once in the morning, and once in the afternoon. The amount of feed provided to the animals was 2.5 kg/animal/day, on average, but could vary according to the body score and age of the animal, which was evaluated by the veterinarian responsible for the SPC. Water was provided ad libitum to the animals.

Following a completely randomized design (CRD), boars were divided into four groups: INOR30 – diet formulated with an inorganic Se source (0.30 ppm sodium selenite), COMP30 – diet with an amino acid complexed source of Se (0.30 ppm of Se metal-amino acid), MIXED15+15 - diet formulated with inorganic and complexed Se (0.15 ppm of sodium selenite + 0.15 ppm of Se metal-amino acid), and COMP15 - diet formulated with low level complexed Se (0.15 ppm metal-Se amino acid) (Table 1). The dose of the metal-amino acid in the diet was defined according to the NRC[11]. In the Se source in the metal-amino acid form, methionine was used as the carrier amino acid.

Table 1.Nutritional composition of diets fed to boars

NUTRIENT	Diet			
	INOR30	COMP30	MIXED15+15	COMP15
Dietary DE (kcal/kg)	3100	3100	3100	3100
Crude protein (min.) (g/kg)	5.0	5.0	5.0	5.0
Ether extract (min.) (g/kg)	30.0	30.0	30.0	30.0
Crude fiber (max.) (g/kg)	40.0	40.0	40.0	40.0
Mineral matter (max.) (g/kg)	80.0	80.0	80.0	80.0
Calcium (g/kg)	8.0 – 10.0	8.0 – 10.0	8.0 – 10.0	8.0 – 10.0
Phosphorus (mg/kg)	6000.0	6000.0	6000.0	6000.0
Sodium (mg/kg)	2400.0	2400.0	2400.0	2400.0
Copper (mg/kg)	145.0	145.0	145.0	145.0
Chromium (mg/kg)	0.40	0.40	0.40	0.40
Iodine (mg/kg)	1.68	1.68	1.68	1.68
Iron (mg/kg)	100.0	100.0	100.0	100.0
Manganese (mg/kg)	55.0	55.0	55.0	55.0
Inorganic selenium (mg/kg)	30.0	0.0	0.15	0.0
Methionine selenium (mg/kg)	0.0	30.0	0.15	0.15
Bacillus subtilis (UFC/kg)	$16x10^5$	$16x10^5$	$16x10^5$	$16x10^5$
Bacillus licheniformis (UFC/kg)	$16x10^5$	$16x10^5$	$16x10^5$	$16x10^5$
Choline (mg/kg)	1.60	1.60	1.60	1.60
Lysine (mg/kg)	8400.0	8400.0	8400.0	8400.0
Methionine (mg/kg)	4000.0	4000.0	4000.0	4000.0
Threonine (mg/kg)	6400.0	6400.0	6400.0	6400.0
Tryptophan (mg/kg)	1750.0	1750.0	1750.0	1750.0
Valine (mg/kg)	5800.0	5800.0	5800.0	5800.0
Vitamin A (UI/kg)	20000.0	20000.0	20000.0	20000.0
Vitamin B1 (mg/kg)	2.67	2.67	2.67	2.67
Vitamin B3 (mg/kg)	26.0	26.0	26.0	26.0
Vitamin B5 (mg/kg)	17.44	17.44	17.44	17.44
Vitamin B7 (mg/kg)	1.00	1.00	1.00	1.00
Vitamin B9 (mg/kg)	2.39	2.39	2.39	2.39

Source: Adapted from the table provided by SPC (2017).

Semen collection and evaluations

The experimental period lasted approximately eight months, with two months being the adaptation period, and 22 weeks of collection. The boars underwent semen collection once a week, on average, but this interval was sometimes shorter due to the central demand for insemination doses, reaching a four-day interval between collections.

The collection of total ejaculates was performed by trained employees, using the gloved hand technique[12]. Before collection, the animals underwent the procedure of dry cleaning the foreskin, emptying the preputial diverticulum, and then proceeded to collection on the dummy. The ejaculate was filtered to separate the liquid and gelatinous fractions of the semen, and placed in a thermal collection cup with water previously heated to 37°C.

After collection, the semen in natura was immediately sent to the Laboratory of the SPC for analysis of volume, motility, pH, and presence of agglutination and, later, fractions of the semen were sent to the Laboratory of Swine Reproduction, belonging to UFPR – Sector Palotina for the analysis of sperm concentration and morphology. For this, an aliquot of in natura semen was added to a buffered formalin-saline solution for concentration and morphology analysis, in the proportion of 1:100 (0.1 mL of in natura semen for 10 mL of solution), and 1:5 (0.1 mL of semen to 0.5 mL of solution), respectively. In total, ejaculates from 35 boars in reproductive activity were evaluated for 22 weeks, resulting in 210 samples evaluated for volume, motility, pH, presence of agglutination, and morphological alterations, and 140 samples for sperm concentration.

Analysis of seminal quality

Volume

The ejaculate volume was measured by weighing the liquid fraction of the semen, in the collection cup itself, disregarding its weight and, after removing the

filter paper, where the gelatinous fraction of the collected semen was. It is accepted that each 1 mL of semen corresponds to 1 g (gram)[13]. Measurement of volume is important to determine the total number of sperm in the ejaculate.

Motility

The reading was performed in a binocular microscope with a 10X objective, using a drop of semen in natura between a slide and a coverslip, previously heated to 37°C. The technique was performed by a technician from the SPC laboratory. This method evaluates the total amount of motile sperm in the sample, classifying this movement in a score from 0 to 100%.

pH

The pH of the semen sample was measured with a pH meter (mPA-210p), which was calibrated every day before collections.

Agglutination

The presence of agglutinated sperm cells was expressed in crosses, being classified from zero to three crosses according to the proportion of agglutinated sperm, zero being the absence of agglutination, and three being intense agglutination of sperm.

Concentration

In the UFPR laboratory, the concentration was analyzed using a Neubauer chamber. A bright-field microscope was used, with a 40 X objective, where five squares were counted on each side of the chamber using the inverted L methodology. The total sperm concentration of the sample, expressed in billions of cells, was calculated by the following formula, described by the Brazilian College of Animal Reproduction (CBRA)[13]:

$$\frac{A}{\frac{1}{B} \times \frac{N}{25} \times \frac{1}{10}} = nº\ of\ espermatozoa/mm^3$$

Wherein: A = number of spermatozoa counted; B = dilution factor; N = number of squares counted; 1/10 = chamber height

Morphological Analysis

The wet preparation technique was used to evaluate the morphological characteristics of each sample. A phase-contrast microscope was used at 1000 X magnification in oil immersion. A drop of the pre-fixed sample, in buffered formaldehyde-saline solution, was placed between a slide and a coverslip, and the sperm morphological evaluation of 200 cells was performed.

The classification of anomalies found was expressed as a percentage. Head defects (small, giant, pear-shaped, and isolated), acrosome, midpiece, tail (bent, bent with a drop, strongly bent, broken, and isolated), proximal cytoplasmic drop, and teratogenic forms were evaluated.

Statistics

For the statistical analysis, the weeks of collections were grouped to obtain smaller amounts of repeated measures over time and to enable the visualization of the comparison of the averages. This condensation of weeks considered that this examination is normally carried out in the centers periodically, following the shortest period of the sperm cycle of the species (35-60 days)[14]. The first two weeks were analyzed together and, subsequently, they were grouped every four weeks, following the order of collection (Table 2).

Table 2. Periods formed by grouping weeks

Grouping	Periods					
	1	2	3	4	5	6
Weeks	1 to 2	3 to 6	7 to 10	11 to 14	15 to 18	19 to 22

The data were analyzed with repeated measures in the time in a mixed model (PROC MIXED) in which the fixed effects of the type of selenium supplementation in the ration (3 degrees of freedom - DF), evaluation periods (5 DF), and their interactions were considered. (15 DF). The age of the animals was included as a covariate, and the *estimating breeding value* (EBV) classes as a block in the model, seeking to control these effects in the variables analyzed. The random effects of the animal and the employee who collected the ejaculates were considered in the model. The most adequate error structure for each variable was defined according to the corrected Akaike (AICc) and Bayesian (BICc) information criteria. The means that showed a significant difference ($P<0.05$) for the fixed effects and their interactions were compared using the Fischer test (PROC LSMEANS). The analyzes were performed using the Statistical Analysis System (SAS) program, version 9.0.

Results

The different sources and doses of Se used showed a similar influence on the evaluated seminal characteristics, and it was possible to verify that the volume, motility, cell agglutination, pH, concentration, and sperm dose did not differ between the groups that received sodium selenite or selenium metal amino acid in doses of 0.15 ppm or 0.30 ppm (Table 3).

Table 3. Characteristics of boar semen receiving different types of selenium supplementation in diets

Variable[a]	Treament[b]				SEM[c]	P value[d]		
	INOR30	COMP30	MIXED15+15	COMP15		Treat	Period	T × P
Vol	272.84	276.77	259.40	270.10	5.92	0.9810	<0.0001	0.1910
Motil	93.56	93.88	93.15	93.94	0.14	0.5602	<0.0001	0.6034
Aggl	1.50	1.50	1.31	1.33	0.07	0.8240	<0.0001	0.8163
pH	7.41	7.34	7.39	7.45	0.01	0.5291	<0.0001	0.1414
Conc	57.01	55.40	56.11	62.80	1.78	0.8863	<0.0001	0.9160
Dose	17.98	17.53	17.63	19.73	0.60	0.8969	<0.0001	0.8906

[a]Vol: volume (mL); Agglut: cell agglutination; Motil: motility (%); Conc: concentration (x10^9 sptz/ejac); Dose: number of doses.

[b]INOR30: inorganic Se-based diet (0.30 ppm sodium selenite); COMP30: Se amino-acid complex-based diet (0.30 ppm Se metal-amino acid); MIXED15+15: inorganic and complexed Se mixed diet (0.15 ppm sodium selenite + 0.15 ppm Se metal-amino acid); COMP15: diet low in complexed Se (0.15 ppm Se metal-amino acid).

[c]SEM: standard error of the mean.

[d]Treat: type of selenium supplementation; T × P: interaction between treatment and period.

There was no interaction between the type of selenium supplementation (sources and doses) and the period of consumption of this mineral on the semen characteristics of boars (Table 3). On the other hand, there was an effect of the supplementation period on the seminal quantity and quality of boars. The number of doses produced decreased with time, even with the increase in seminal quality over the 22 weeks evaluated, due to the reduction in sperm concentration (Tables 4 and 6).

In Table 4, it is possible to verify that the volume varied during the observed period, with a lower volume ($p<0.05$) between weeks 7 and 10, and increasing again in subsequent weeks. On the other hand, sperm concentration showed a reduction in cellularity from the 15th week of evaluation. Motility improved progressively, with a difference ($p<0.05$) from the seventh week of evaluation compared with the previous weeks. The presence of agglutination in the ejaculates was greater over time, with an increase of more than three times being observed in the last two periods evaluated concerning the first observation. The pH of the samples varied in the experimental period.

Table 4. Characteristics of boar semen as a function of time of supplementation with different sources and doses of selenium

Weeks	Variable[a]					
	Vol	Motil	Agglut	pH	Conc	Dose
1 to 2	277.24^{ab}	92.89^{b}	0.64^{d}	7.35^{d}	-	-
3 to 6	270.10^{ab}	92.46^{b}	0.89^{cd}	7.42^{b}	-	-
7 to 10	247.32^{c}	93.81^{a}	1.08^{c}	7.39^{c}	61.17^{a}	19.28^{b}
11 to 14	263.95^{b}	94.21^{a}	1.63^{b}	7.46^{a}	67.55^{a}	21.40^{a}
15 to 18	284.18^{a}	94.38^{a}	2.07^{a}	7.41^{bc}	49.29^{b}	15.53^{c}
19 to 22	275.88^{ab}	94.05^{a}	2.16^{a}	7.35^{d}	53.30^{b}	16.66^{c}

[a]Vol: volume (mL); Agglut: cell agglutination; Motil: motility (%); Conc: concentration (x10^9 sptz/ejac); Dose: number of doses.

Means followed by different lowercase letters in the columns differ from each other by the F-test ($p<0.05$).

The morphological parameters were also not influenced by the different sources and levels of selenium supplementation, however, there was an effect of the period, as observed in the other analyzes (Table 5). When evaluating isolated sperm defects, it is possible to verify that the presence of proximal cytoplasmic droplet and tail defects are the alterations found in greater proportion, representing approximately 20% of morphological defects, as observed in the MIXED15+15 group.

Table 5. Morphological characteristics of spermatozoa in the semen of boars receiving different selenium supplementation types in the diet

Variable[a] (%)	Treatment[b]				SEM[c]	P-value[d]		
	INOR30	COMP30	MIXED15+15	COMP15		Treat	Period	T × P
Normal	85.18	82.98	76.28	81.68	1.01	0.4748	<0.0001	0.1410
PCD	6.19	11.87	12.68	9.29	0.94	0.6903	0.0074	0.2843
TD	6.25	2.32	6.64	6.42	0.34	0.2500	<0.0001	0.3133
HD	0.97	1.48	2.68	1.76	0.20	0.2479	0.0071	0.4025
AD	0.36	0.63	0.14	0.16	0.04	0.4804	0.0007	0.3368
MPD	0.34	0.63	0.96	0.41	0.06	0.2427	0.0091	0.7136
Teratog	0.03	0.02	0.02	0.03	0.01	0.9207	0.1478	0.8267

[a]PCD: proximal cytoplasmic droplet; TD: tail defect; HD: head defect; AD: acrosome defect; MPD: midpiece defect; Teratog: teratogenicity.

[b]INOR30: inorganic Se-based diet (0.30 ppm sodium selenite); COMP30: Se amino-acid complex-based diet (0.30 ppm Se metal-amino acid); MIXED15+15: inorganic and complexed Se mixed diet (0.15 ppm sodium selenite + 0.15 ppm Se metal-amino acid); COMP15: diet low in complexed Se (0.15 ppm Se metal-amino acid).

[c]SEM: standard error of the mean..

[d]Treat: type of selenium supplementation; T × P: interaction between treatment and period.

Observing the evolution of morphological changes over the 22 weeks of study, it is possible to verify an improvement in the parameters evaluated. In Table 6 it is possible to verify that the index of normal sperm cells was higher in the last period evaluated, with fewer defects in the tail, head, acrosome, and intermediate piece, especially from the third month of analysis, referring to the fifth month of boar supplementation. Only the index of the presence of proximal cytoplasmic droplets (PCD) worsened in the period evaluated.

Table 6. Morphological characteristics of spermatozoa in the semen of boars as a function of time of supplementation with different sources and doses of selenium

Weeks	Variable[a] (%)						
	Normal	PCD	TD	HD	AD	MPD	Teratog
1 to 2	78.59^{d}	8.50^{b}	7.16^{a}	3.73^{a}	0.62^{a}	0.99^{a}	0.00
3 to 6	83.16^{ab}	9.52^{ab}	4.70^{bc}	1.36^{bc}	0.33^{b}	0.45^{b}	0.03
7 to 10	80.14^{cd}	10.97^{a}	5.62^{b}	1.71^{b}	0.33^{b}	0.75^{a}	0.03
11 to 14	81.71^{bc}	10.24^{a}	5.53^{b}	1.50^{bc}	0.22^{cd}	0.44^{b}	0.03
15 to 18	81.33^{bc}	10.69^{a}	5.91^{ab}	1.09^{bc}	0.14^{d}	0.41^{b}	0.02
19 to 22	84.27^{a}	10.13^{a}	3.51^{c}	0.97^{c}	0.28^{bc}	0.46^{b}	0.04

[a]PCD: proximal cytoplasmic droplet; TD: tail defect; HD: head defect; AD: acrosome defect; MPD: midpiece defect; Teratog: teratogenicity.
Means followed by different lowercase letters in the columns differ from each other by the F-test ($p<0.05$).

Discussion

Our findings are similar to those of Lovercamp et. al.[15], in which swine diet supplementation with different Se sources did not affect semen quality. On the other hand, the half dose of Se complexed with amino acid (0.15 ppm) provided the same effect as the commonly used sodium selenite dose (0.30 ppm). This result corroborates several studies that indicate that a Se organic source has greater use than the inorganic one due to an increased bioavailability of organic minerals [9, 10, 16, 17].

Coordinate and ionic bonds formed by amino acid ligands protect the mineral, preventing physical-chemical factors from interfering with its absorption. After being absorbed, amino acids pass directly into plasma through

intestinal mucosa cells, carrying the mineral that remains bound to amino acids[10,18, 19]. As a result, metal-amino acids become more bioavailable and bioactive [10,17]; therefore, they can be included in diets at lower levels than inorganic minerals, without compromising animal performance. This addition also minimizes environmental impacts since the metal is less excreted into the environment[17]. This finding corroborates the latest publication by Rostagno et al.[20], who suggested that inorganic Se should be included in boar diets at a dose of 0.40 ppm, while Se complexed with an organic source should have its inclusion reduced to 0.18 ppm, demonstrating the lower need for Se from organic sources.

Spermatic volume and concentration are the most evaluated parameters in SPC to calculate insemination doses. These parameters are affected by boar maturity: with aging, ejaculate total volume increases, while its concentration decreases[21]. According to Smital[21], who evaluated more than 230 thousand ejaculate records, spermatic cell production varies with boar's age and breed, increasing considerably within the first three years, reaching a maximum of 3.5 years, and declining thereafter. The author also showed that seminal recovery is related to boar mating intensity, requiring 5 to 7 days to restore ejaculate total volume and up to 11 days for the complete restoration of spermatic concentration. In our experiment, animals were relatively young, averaging 2 years old at the beginning of the study. Therefore, spermatic concentration was expected to increase, unlike what was observed. Instead, the number of spermatozoa declined over the 22 weeks evaluated. This result suggests that boars were in intense mating activity, without time to fully recover their spermatic concentration.

In swine, ejaculate volume can vary between 125 and 500 mL according to breed, age, and sampling frequency[22]. In our study, the mean volume was 250 mL and, despite varying throughout the experiment, it remained within the normal range for the species. The same occurred with ejaculate pH, which varied in the experimental period but remained within the normal range for the species that is slightly alkaline, ranging from 7.3 to 7.9[13].

Cell agglutination in swine ejaculate is relatively common and can be observed in almost every sample examined. It can be induced by dead sperm or epithelial cells, bacterial contamination, or fast cooling. Such a reaction can be visualized by the union of sperm heads during sperm motility assessment[22]. In our study, agglutination increased gradually and coincided with an increase in cytoplasmic droplets in samples. The latter could have been a predisposing factor for the agglutination increase. However, no reports were found in the literature correlating these variables.

We observed spermatic motility above 90%, which is acceptable according to CBRA[13], which suggests a minimum percentage of 70%. Sperm motility improved over 22 weeks of assessment, increasing by 1.16% between the first and last assessment period. Petrujkic et al.[23] observed increases in motility and fertility rate for boars fed Se complexed with an organic source. Likewise, Moslemi and Tavanbakhsh[8] observed an increase in the spermatic motility of subfertile human patients supplemented with Se and vitamin E. These authors noted that motility was the parameter most correlated with fertility, including cases in which the oral administration of these two antioxidants resulted in fertilization.

When evaluating morphological changes, it is expected that the number of abnormal cells does not exceed 20% of the total number of cells evaluated [13]; however, the group MIXED15+15 was slightly lower than expected (76.28%), but did not differ statistically from the other groups.

By evaluating sperm defects in isolation, only PCD increased over time, while the others decreased. The average of PCD was within the acceptance limit for the swine species assessed, which is up to 10% of defects of this order[13], reaching an average of 12.68% in the group MIXED15+15.

The presence and location of a cytoplasmic droplet (CD) reflect the stage of sperm maturation and can cause fertility problems, impairing production[24]. Distal cytoplasmic drop (DCD) is normally detached from the sperm tail during ejaculation, which may be a result of low collection frequency, while PCD is detected in young males or adult breeding males in high mating activity, or even in pathological cases [22]. Marin-Guzman et al.[7] suggested that Se speeds up sperm maturity in the epididymis and may reduce the number of sperm cells with CD, unlike what was observed in our study, in which the index of spermatozoa with PCD increased over time.

In our study, only PCD was evaluated as a morphological defect, while DCD was counted as a normal sperm cell, according to CBRA[13]. This orientation is because DCD is not considered a defect, therefore, it would not cause reproductive harm [13]. Conversely, Gaggini et al.[24] observed that DCD influences sperm motility, impairing the fertility of breeding males. Thus, CD values in evaluated ejaculates would be even higher if the presence of DCD were included in counts.

By evaluating sperm tail defects, we could observe that the group supplemented with 0.30 ppm Se metal-amino acid (COMP30) had almost three times fewer tail defects (2.32%) than the other groups (mean of 6.44%). Despite this, there were no statistical differences among them. Such a result may be due to the high standard deviation of the analyzed data, which was 4.78 and 6.70 percentage points (plus or minus) on the average. Even so, this result may indicate that Se in its complexed form had

a greater protective potential for the cell against oxidative damage since the lipid membrane constituting spermatozoa is rich in polyunsaturated fatty acids, which need an antioxidant system to maintain their integrity[25, 26].

Our findings demonstrate that, regardless of the source used, Se demands seem to have been met to maintain seminal quality. In other species, a relationship between Se and seminal quality of males with low fertility has been reported in the literature[27,28]. In swine breeding, boars with low fertility parameters are immediately excluded. However, no studies have evaluated the influence of Se supplementation on improved fertility in boars. This may be a line of research to be established since swine breeders have a high impact on pig farming, and diets with organic Se increase semen antioxidant capacity but do not influence the seminal quality, as demonstrated by Martins et al.[29]. This might be because there are no challenges of this order that require increases in Se inclusion in pig diets.

Conclusion

The inclusion of half the dose of selenium complexed with methionine produces the same effect on the seminal quality of boars compared to a diet with sodium selenite at the dose recommended by the nutritional tables for swine breeding.

Conflict of interest

The authors declare no conflict of interest.

Authors' Contributions

Conceptualization: A. Teixeira, D. G. Donin, A. F. K. Nogueira, G. C. Alberton; *Data curation:* A. Teixeira, S. R. Fernandes; *Formal analysis:* S. R. Fernandes; *Acquisition of financing:* A. F. K. Nogueira; Research: A. Teixeira, B. Zuffo, A. P. Backes, A. J. S. Silva, A. L. Waltrich; *Methodology:* D. G. Donin, G. C. Alberton, A. F. K. Nogueira; *Project management:* A. Teixeira, D. G. Donin; *Resources*: A. Teixeira, A. F. K. Nogueira; *Supervision:* G. C. Alberton, D. G. Donin; *Validation:* D. G. Donin, G. C. Alberton, S. R. Fernandes, A. F. K. Nogueira; *View:* A. Teixeira, D. G. Donin, D. G. Donin, S. R. Fernandes; *Writing:* A. Teixeira, D. G. Donin, D. G. Donin, S. R. Fernandes.

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