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Effect of supplementation with selenium proteinate and sodium selenite on performance, digestibility, intestinal integrity, and expression of selenoproteins in weaned piglets

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ABSTRACT - The study was carried out with the objective of investigating the enrichment of weaned piglet diets with selenium proteinate (SeP) and sodium selenite (SeS) at two inclusion levels. A total of 40 piglets, averaging 6.60 ± 1.06 kg, were assigned to five dietary treatments: a control diet, and diets supplemented with selenium proteinate (0.15 or 0.30 mg/kg) or sodium selenite (0.15 or 0.30 mg/kg). The SeP-0.30 diet reduced average daily feed intake (ADFI) ($P<0.05$) in both periods and average daily gain (ADG) ($P = 0.017$) in the second period. Diets with selenium proteinate improved the digestibility of dry matter (DM; $P = 0.024$) and organic matter (OM; $P = 0.024$). All selenium-enriched diets increased selenium retention ($P<0.05$). The addition of 0.30 mg/kg of selenium reduced villus height (VH) ($P = 0.001$), villus width (VW) ($P = 0.001$), crypt depth (CD) ($P<0.0001$), and mitotic rate ($P = 0.031$). Sodium selenite reduced apoptosis in the jejunal epithelium ($P = 0.020$) and increased hepatic selenoprotein P (SePP) expression ($P = 0.038$). High concentrations of sodium selenite elevated hepatic selenoproteins, serum creatinine (CRC), and Immunoglobulin A (IgA) concentrations. Supplementation with sodium selenite should not exceed 0.30 mg/kg, whereas selenium proteinate is recommended at up to 0.15 mg/kg. Increasing selenium, particularly as proteinate, yields positive results, but levels above 0.30 mg/kg may be detrimental.

Keywords: antioxidant, immunity, oxidation process, serum parameters

1. Introduction

Selenium is an essential trace element for pigs. It contributes to immune function, enhances antioxidant activity, and helps prevent conditions such as muscular dystrophy, exudative diathesis, necrotic

degeneration of the liver and mulberry heart pathology (Żarczyńska et al., 2013; Cao et al., 2014; Rao et al., 2023). This is particularly relevant in the post-weaning phase, when selenium requirements are higher than in subsequent phases NRC (2012). In this sense, meeting the selenium requirement is necessary to avoid pathologies and maintain redox balance.

Selenium has one of the narrowest margins between dietary deficiency (<40 µg/day) and toxicity levels (>400 µg/day) of all the elements (WHO, 1996), especially when supplemented through inorganic sources such as sodium selenite. The selenium requirement recommended by the NRC (2012) for piglets is 0.300 mg/kg, and Rostagno et al. (2011) recommend 0.410 mg/kg of organic source for post-weaning piglets. However, Rostagno et al. (2017) recommend 0.517 mg/kg of inorganic source and 0.233 mg/kg of organic source for post-weaning piglets. Therefore, studies comparing selenium sources and inclusion levels are warranted.

Various organic selenium sources, such as selenium-enriched yeast, selenium proteinate, and selenium-amino acid, have been introduced to the animal feed industry. Other organic selenium sources have been developed to improve the bioavailability of Se products in animals and selenium proteinate is one of these organic Se sources which is produced using enzymatically hydrolyzed soy protein (Jang et al., 2010). The source and inclusion level of selenium are critical for animal nutrition, as they influence muscle selenium deposition (Zoidis et al., 2014; Zhang et al., 2020), typically show greater bioavailability for organic than inorganic sources (Lyons et al., 2007; Rao et al., 2023).

Therefore, this study was conducted with the objective of investigating the enrichment of diets for weaned piglets using selenium proteinate and sodium selenite and two addition levels (0.15 and 0.30 mg/kg) on productive performance and metabolism.

2. Material and methods

The experimental test was carried out at the Pig Farming Laboratory of the Department of Animal Science at the Center for Human, Social and Agrarian Sciences at the Universidade Federal da Paraíba in Bananeiras, PB, Brazil (6°45'12.9" South latitude, 35°38'57.5" West longitude, and average altitude of 552 m). The experimental procedures were approved by Ethics Committee on the Use of Animals of the Universidade Federal da Paraíba (CEUA/UFPB), under experimental protocol number 4328201218.

2.1. Animals and experimental diets

A total of 40 piglets (20 castrated males and 20 females) weaned at 28 days of age, with an average initial body weight of 6.60±1.06 kg, and all from the same commercial strain (Topigs) were used. The number of animals used followed the guidelines of the CEUA/UFPB. Sample size was determined according to Sakomura and Rostagno (2016). Piglets did not receive additional selenium supplementation during the pre-weaning phase.

The pigs were housed in suspended nursery cages with a perforated plastic floor, equipped with nipple drinkers and semi-automatic feeders.

Animals were allocated in a randomized block design with five dietary treatments and four replicates, with the experimental unit consisting of two pigs (one male and one female). The dietary treatments were as follows: control diet containing selenium from the mineral supplement (control diet); control diet enriched with selenium proteinate with 0.15 mg/kg of selenium added (SeP-0.15); control diet enriched with selenium proteinate with 0.30 mg/kg of selenium added (SeP-0.30); control diet enriched with sodium selenite with 0.15 mg/kg of selenium added (SeS-0.15); and control diet enriched with sodium selenite with 0.30 mg/kg of selenium added (SeS-0.30). The basal selenium in the control diet was derived from the mineral supplement (Table 1).

The experimental diets, except for selenium concentration, were formulated according to the recommendations of Rostagno et al. (2017) for three phases: I - from 28 to 35 days of age; II - from 36 to 45 days of age; III - from 46 to 60 days of age (Table 2).

Table 1 - Levels of selenium concentrations in the experimental diets[1]

Treatment[2]	Growing phase		
	I (28-35 days)	II (36-45 days)	III (46-60 days)
Control diet	0.276	0.265	0.254
SeP-0.15	0.426	0.415	0.404
SeP-0.30	0.576	0.565	0.554
SeS-0.15	0.426	0.415	0.404
SeS-0.30	0.579	0.568	0.557

[1] Selenium proteinate was used (YesSinergy, São Paulo, Brazil) and sodium selenite (Dinâmica, Indaiatuba, Brazil) as sources of selenium.
[2] Control diet - Control diet containing selenium from mineral supplement; SeP-0.15 - Control diet enriched with selenium proteinate with 0.15 mg/kg of selenium added; SeP-0.30 - Control diet enriched with selenium proteinate with 0.30 mg/kg of selenium added; SeS-0.15 - Control diet enriched with sodium selenite with 0.15 mg/kg of selenium added; SeS-0.30 - Control diet enriched with sodium selenite with 0.30 mg/kg of selenium added.

Table 2 - Ingredients and chemical composition (g/kg, as feed) of the experimental diets

Ingredient	28-35 days	36-45 days	46-60 days
Corn	457.8	529.2	700.2
Soybean meal	327.6	291.0	239.0
Whey powder	120.0	80.0	0.0
Soy oil	43.4	40.9	9.5
Dicalcium phosphate	19.6	18.8	17.3
Limestone	9.90	8.93	7.04
L-lysine HCl	5.46	5.56	4.49
DL-methionine	2.40	2.23	1.19
L-threonine	3.06	2.94	1.72
L-tryptophan	0.55	0.57	0.39
L-arginine	2.30	2.23	0.00
L-valine	1.37	1.31	0.41
Vitamin supplement[1]	2.00	2.00	2.00
Mineral supplement[2]	1.00	1.00	1.00
Salt	2.73	3.48	4.72
Inert[3]	1.00	9.95	11.00
Calculated values			
Metabolizable energy (MJ/kg)	14.23	14.12	13.60
Crude protein	214.20	198.70	176.90
Digestible lysine	14.51	13.46	10.97
Digestible methionine + cystine	8.13	7.54	6.25
Digestible threonine	9.72	9.02	7.13
Digestible tryptophan	2.76	2.56	2.08
Digestible arginine	14.51	13.46	10.19
Digestible valine	10.01	9.29	7.60
Limestone	10.68	9.73	7.94
Available phosphorous	5.28	4.81	3.93
Sodium	2.24	2.19	1.99
Potassium	9.97	8.69	6.62

[1] Vitamin A, 4,000 IU; vitamin D3, 220 IU; vitamin E, 22 mg; vitamin K, 0.5 mg; vitamin B2, 3.75 mg; vitamin B12, 20 mg; calcium pantothenate, 12 mg; niacina, 20 mg; choline, 400 mg.
[2] Iodine, 140 µg; selenium, 300 µg; manganese, 10 mg; zinc, 100 mg; copper, 10 mg; iron, 99 mg.
[3] The inclusion of proteinate selenium and sodium selenite was carried out in partial replacement of the inert. In the third phase, the inert was partially replaced by Celite 545, which is the internal marker of acid insoluble ash (AIC) for carrying out the apparent digestibility.

2.2. Productive performance and fecal score

The pigs and feed refusals were weighed at the beginning and end of each experimental phase to calculate average daily feed intake (ADFI), average daily gain (ADG) and feed conversion (FC) of the weaned piglets. Growth performance was evaluated for the periods from 28 to 45 days of age and from

28 to 60 days of age. The fecal score was evaluated from 28 to 45 days as an indication of diarrhea incidence. Fecal scores were recorded twice daily in the morning and in the late afternoon. Stool consistency was evaluated according to visual criteria, in which: 1, normal; 2, pasty; and 3, liquid; a score of 3 was considered as diarrheal stools following the method proposed by Pascoal et al. (2012).

2.3. Digestibility of the diets

Apparent digestibility of nutrients and energy in the diets was evaluated during the third experimental phase. To this end, 1% of CELITE 545 (Diadema, SP, Brazil) was added to the diets as an internal marker and an acid-insoluble ash (AIA) source replacing the inert source. The assay lasted 7 days, with the first three days for adaptation and the last four days for feces collection. At the end, the feces of each pig were homogenized and dried at 55 °C for 72 hours. Feed and feces samples were ground and passed through a 0.5 mm sieve, then analyzed according to the procedures described by (AOAC, 2006) for determining dry matter (DM), mineral matter (MM), organic matter (OM) and crude protein. Gross energy was determined using an adiabatic bomb calorimeter (6100 model, Parr Instruments Co., San Francisco, CA, USA). Selenium concentration was determined by hydride generation atomic absorption spectrometry (iCE 3500 model, HGAAS ICE 3000 Series Thermo Scientific device, Cambridge, UK) coupled to a UP 100 hydride regenerator. Analysis employed Solar software program, an air-acetylene flame and a selenium hollow cathode lamp (Photoron-Lamps-Pty-Ltd, Victoria, Australia). Measurements were performed at 196 nm, with a calibration ranging from 5 to 40 mg/L. Total tract apparent digestibility coefficients (TTADC) of nutrients and energy were calculated according to Adeola (2000), as follows:

$$\text{TTADC} = 1 - \frac{\text{AIA concentration in the diet} \times \text{Component in the feces}}{\text{AIA concentration in the feces} \times \text{Component in the diet}}$$

The retained selenium and excreted selenium were calculated as follows:

$$\text{Se retained} = \text{Se in diet} - \left(\text{Se in feces} \times \frac{\text{AIA concentration in the diet}}{\text{AIA concentration in the feces}}\right)$$

$$\text{Se excreted} = \text{Se in the diet} - \text{Se retained}$$

2.4. Sampling and processing

Blood samples were collected from the jugular vein of one piglet per experimental unit at 41 and 60 days of age, selected according to the average weight of each treatment. Serum was separated by centrifugation at 1006 × *g* for 10 minutes and transferred to microtubes (Eppendorf, São Paulo, Brazil). At the end of the experimental period, piglets were slaughtered after a 12-hour fast by electrical stunning followed by exsanguination in accordance with the procedures approved by CEUA/UFPB. Segments of the proximal duodenum portion and the mid-jejunum were collected after slaughter and immediately immersed in metacarn solution for histological and immunohistochemical evaluations. Samples were dehydrated in increasing alcohol solutions, cleared in xylene and embedded in paraffin according to the protocol described by Yoon et al. (2012). Muscle samples (*Longissimus thoracis*) and blood were collected for selenium analysis, following the methodology used for selenium determination in diet and fecal samples. Jejunum and muscle (*Longissimus thoracis*) samples were collected to determine Malondialdehyde concentration, and jejunum and liver samples were collected for gene expression analysis. Samples for malondialdehyde and gene expression analysis were stored at −80 °C until further analysis.

2.5. Intestinal morphometry and integrity

Histological slides were stained with hematoxylin and eosin to assess intestinal morphometry. Villus height (VH), villus width (VW) and crypt depth (CD) were measured, and the villus height:crypt

depth ratio (VW:CD) was calculated. Cell death by apoptosis (Caspase) and nuclear protein proliferation nuclear antigen (PCNA-rate of mitosis) were determined using the protocol used for all antibodies based on the immunohistochemistry technique (Terzian et al., 2007). The apoptosis rate in villous cells of the mid-jejunum was assessed by anti-neutrophil cytoplasmic antibody positivity. Positivity scores were assigned to the 20 photomicrographs per treatment using the following scale: 0 (absence of positivity), 1 (low positivity), 2 (mild positivity) and 3 (intense positivity), with methodology being adapted from (Ishak et al., 1995). Crypts were randomly analyzed and measured to assess the cell mitosis rate in the first duodenum portion and the middle jejunum portion, making a total of 10,000 µm of epithelium per treatment. Epithelial cells proliferation was quantified based on the number of anti-PCNA-positive nuclei. Slides were analyzed and digitized using a light microscope (Olympus BX53, 40× objective, Tokyo, Japan) coupled to a Zeiss Axio camera (Oberkochen, Germany) and a computer equipped with CellSens Dimension software (Olympus, Tokyo, Japan).

2.6. Biochemical determinations

Serum concentration of immunoglobulin A (IgA), immunoglobulin G (IgG), gamma glutamyl transferase (GGT), aspartate aminotransferase (AST) and creatinine (CRC) were determined spectrophotometrically using commercial kits (Labtest Diagnóstica, Lagoa Santa, Minas Gerais, Brazil): IgA (Turbiquest, ref. 358), IgG (Turbiquest, ref. 359), GGT (Liquiform, ref. 105), AST (Liquiform, ref. 109) and creatinine (CRC). Thiobarbituric acid reactive substances (TBARS) were determined according to Rosmini et al. (1996). Briefly, 1.0 mL of 0.5% sulfanilamide solution, 10 mL of 10% (v/v) trichloroacetic acid (TCA) solution and 5.0 mL of distilled water were added in a tube containing 5 g of sample. The mixture was shaken for five minutes to extract malonaldehyde (MDA) and centrifuged for 5 minutes at 1006 × *g*. The supernatant was filtered (Whatman qualitative filter paper, Grade 1) and mixed with 5 mL of 0.02 M thiobarbituric acid (TBA). The mixture was then heated in a water bath at 100 °C for 35 min. The tubes were subsequently cooled to room temperature (25 °C) in an ice bath. Finally, the absorbance reading at 532 nm was performed in a spectrophotometer (UV-V AKSO N6000Plus, USA).

2.7. Gene expression

Samples from the mid-jejunum and liver were collected for gene expression analysis by quantitative real-time PCR (qPCR). Total RNA was extracted from the samples using the Qiagen RNeasy Mini kit (Cat. N.74106) and cDNA synthesis was performed using the cDNA High Capacity cDNA Reverse Transcription kit (Applied Biosystems), according to the manufacturers' recommendations. Relative gene expression was determined by qPCR using Power SYBR Green Master Mix (Thermo Fisher Scientific, Applied Biosystems) and gene-specific primers (Table 3) for glutathione peroxidase 1 (GPX1), glutathione peroxidase 2 (GPX2), glutathione peroxidase 4 (GPX4), and selenoprotein P (SePP). The reference genes β-actin (ACTB) and glyceraldehyde-3-phosphate dehydrogenase (GAPDH) were used for normalization.

Table 3 - Genes and sequence of primers used in qPCR

Gene	Forward Primer sequences	Reverse primers sequences	PS[1]	Locus[2]
GPX1	gctcggtgtatgccttctct	agcgacgctacgttctcaat	103	AF532927
GPX2	cgtgaatggtcagaatgagc	gggatcagtcatgagggaaa	94	DQ898282
GPX4	attctcagccaaggacatcg	cctcattgagaggccacatt	93	NM_214407
SEPP	gctccttctgtgagcaacct	gcctgaagaagagcaaccac	97	EF113596
ACTB	tgttcgagaccttcaacacg	atccccagagtccatgacaa	104	DQ845171
GAPDH	acatggcctccaaggagtaaga	gatcgagttggggctgtgact	106	AF017079

GPX1 - glutathione peroxidase 1; GPX2 - glutathione peroxidase 2; GPX4 - glutathione peroxidase 4; SePP - selenoprotein P; ACTB - actin beta; GAPDH - glyceraldehyde 3-phosphate dehydrogenase.
[1] Product size.
[2] GenBank.

The qPCR cycles were performed in a thermocycler and the relative expression calculated based on the $2^{-\Delta\Delta Ct}$ method (Livak and Schmittgen, 2001) using the reference genes for expression normalization.

2.8. Statistical analysis

A two-way analysis of variance (ANOVA) was performed to determine the effects of selenium source (SeP or SeS) and addition level (0.15 or 0.30 mg/kg) and the interaction between both factors (source × level). Differences between group means were assessed using Student's *t*-test. A contrast between the control diet and the other diets was estimated by Dunnett's test. Data were processed using the SAS statistical software (OnDemand for Academics). Effects were considered significant at P≤0.05.

The proposed statistical model was as follows:

$$Y_{ijkl} = \mu + \alpha_i + \beta_j + (\alpha\beta)_{ij} + c_k + \varepsilon_{ijkl}$$

in which Y_{ijkl} = value observed in the i-th selenium source, in the j-th additional level of selenium, in the k-th additional treatment and in the l-th replicate; μ = overall mean of the experiment; α_i = fixed effect of factor i (i = 1, 2); β_j = fixed effect of factor j (j = 1, 2); $(\alpha\beta)_{ij}$ = fixed effect of the interaction between factor i (i = 1, 2) and factor j (j = 1, 2); c_k = effect of contrast between the control diet and the other diets k (k = 1); and ε_{ijkl} = random error associated with each Y_{ijkl} observation.

3. Results

Selenium supplementation influenced feed intake and feed conversion (Table 4). There was an interaction effect (P = 0.018) between selenium source and inclusion level for the ADFI variable in both periods; pigs fed the diet containing 0.30 mg/kg of additional selenium proteinate showed lower ADFI. The addition of 0.15 mg/kg of selenium in the diets promoted higher ADFI (P = 0.006) and ADG (P = 0.017) between 28 and 60 days (P<0.05), regardless of the source. Supplementation with selenium

Table 4 - Average daily feed intake (ADFI), average daily gain (ADG) and feed:gain ratio (F:G) of piglets fed diets enriched with proteinate selenium (SeP) or sodium selenite (SeS) at two addition levels (0.15 or 0.30 mg/kg)

	28-45 days			28-60 days		
	ADFI (kg/day)	ADG (kg/day)	F:G (kg/kg)	ADFI (kg/day)	ADG (kg/day)	F:G (kg/kg)
Dietary treatment[1]						
Control diet	0.353	0.199	1.780	0.611	0.396	1.545
SeP-0.15	0.371Aa	0.204	1.821	0.669Aa	0.424	1.580
SeP-0.30	0.330Bb	0.186	1.780	0.562Bb	0.345	1.632
SeS-0.15	0.349Aa	0.198	1.759	0.620Aa	0.410	1.512
SeS-0.30	0.360Aa	0.222	1.625	0.597Aa	0.386	1.549
Dietary Se source[2]						
SeP	0.351	0.195	1.800	0.616	0.384	1.606A
SeS	0.355	0.210	1.692	0.609	0.398	1.530B
Dietary Se level[2]						
0.15 (mg/kg)	0.360	0.201	1.790	0.645	0.417a	1.546
0.30 (mg/kg)	0.345	0.204	1.702	0.580	0.365b	1.590
ANOVA (P-value)						
Dietary Se source	0.651	0.337	0.159	0.719	0.449	0.052
Dietary Se level	0.132	0.854	0.855	0.006	0.017	0.216
Interaction	0.018	0.207	0.964	0.046	0.158	0.633
Pooled SEM	0.007	0.008	0.051	0.013	0.017	0.019

SEM - standard error of mean.
[1] A,B - Differentiate selenium sources at each level (SeP-0.15 X SeS0.15 and SeP-0.30 X SeS-0.30). a,b - Differentiate selenium levels in each source (SeP-0.15 X SeP-0.30 and SeS-0.15 X SeS-0.30).
[2] A,B - Differentiate selenium sources. a,b - Differentiate selenium levels.

proteinate resulted in poorer FC (P = 0.052) in the period from 28 to 60 days of age when compared to sodium selenite. Fecal score was not influenced by selenium source (P = 0.692), addition level (P = 0.909) or interaction between factors (P = 0.472).

Selenium source influenced the apparent digestibility coefficients of DM(P = 0.024) and ash (MM; P = 0.021), with selenium proteinate promoting higher digestibility than sodium selenite. Compared with the control diet, diets supplemented with inorganic selenium at 0.15 and 0.30 mg/kg showed lower DM digestibility (P<0.05; Table 5).

Table 5 - Coefficients of apparent total tract digestibility (CATTD) of the nutrients and energy of diets enriched with proteinate selenium (SeP) or sodium selenite (SeS) at two addition levels (0.15 or 0.30 mg/kg)

	Dry matter	Mineral matter	Protein	Energy
Dietary treatment				
Control diet	0.939	0.499	0.781	0.802
SeP-0.15	0.931	0.379	0.712	0.763
SeP-0.30	0.935	0.409	0.742	0.784
SeS-0.15	0.922*	0.543	0.667*	0.740*
SeS-0.30	0.923*	0.434	0.731	0.779
Dietary Se source[1]				
SeP	0.933A	0.394B	0.727	0.774
SeS	0.922B	0.489A	0.699	0.760
Dietary Se level				
0.15 (mg/kg)	0.926	0.461	0.689	0.751
0.30 (mg/kg)	0.929	0.422	0.737	0.782
ANOVA (P-value)				
Dietary Se source	0.024	0.021	0.275	0.386
Dietary Se level	0.516	0.275	0.080	0.077
Interaction	0.676	0.073	0.507	0.571
Pooled SEM	0.002	0.018	0.013	0.008

SEM - standard error of mean.
[1] A,B - Differentiate selenium sources.
* Dietary treatments that differ from the control diet by Dunnett's test (P<0.05).

Selenium supplementation affected selenium retention, with an interaction effect observed (P = 0.040). Diets supplemented with selenium proteinate or sodium selenite showed higher retention at 0.30 mg/kg. When 0.15 mg/kg of selenium was added, the inorganic source of selenium promoted greater retention compared to organic selenium. In contrast analyses, the control diet resulted in lower selenium retention (P<0.05) than the other treatments. Diets with 0.30 mg/kg of selenium promoted greater excretion (P = 0.031) compared with diets containing 0.15 mg/kg. Compared with the control diet, diets with 0.30 mg/kg selenium proteinate and diets with sodium selenite at 0.15 and 0.30 mg/kg showed higher selenium excretion. Muscle selenium concentration was higher in pigs fed sodium selenite (P = 0.002) and at the 0.30 mg/kg level (P = 0.015). On the other hand, the control diet resulted in lower muscle selenium concentration compared with the diet containing 0.30 mg/kg sodium selenite (Table 6).

The addition of 0.30 mg/kg of selenium to diets resulted in lower VH (P = 0.001), VW (P = 0.001), CD (P<0.0001) and mitotic rate (P = 0.031) in the duodenum. Compared with the control diet, diets with either selenium proteinate or sodium selenite at 0.30 mg/kg reduced VW (P<0.05). In addition, the 0.30 mg/kg selenium proteinate diet reduced mitotic rate (P<0.05), with fewer PCNA-positive cells in the jejunum (Table 7).

Table 6 - Selenium retained, selenium excreted in feces, and selenium present in muscle and blood of piglets fed diets enriched with proteinate selenium (SeP) or sodium selenite (SeS) at two addition levels (0.15 or 0.30 mg/kg)

	Selenium (mg/kg)			
	Retained	Excreted	Muscle	Blood
Dietary treatment[1]				
Control diet	0.232	0.033	0.139	0.117
SeP-0.15	0.361Ab*	0.043	0.131	0.121
SeP-0.30	0.469Aa*	0.085*	0.144	0.135
SeS-0.15	0.326Bb*	0.078*	0.143	0.119
SeS-0.30	0.469Aa*	0.089*	0.186*	0.147
Dietary Se source[2]				
SeP	0.415	0.064	0.138B	0.128
SeS	0.397	0.083	0.165A	0.133
Dietary Se level[2]				
0.15 (mg/kg)	0.344	0.061b	0.137b	0.120
0.30 (mg/kg)	0.469	0.087a	0.165a	0.141
ANOVA (P-value)				
Dietary Se source	0.023	0.074	0.002	0.353
Dietary Se level	<0.0001	0.031	0.015	0.331
Interaction	0.040	0.114	0.191	0.461
Pooled SEM	0.021	0.006	0.005	0.004

SEM - standard error of mean.
[1] A,B - Differentiate selenium sources at each level (SeP-0.15 X SeS-0.15 and SeP-0.30 X SeS-0.30). a,b - Differentiate selenium levels in each source (SeP-0.15 X SeP-0.30 and SeS-0.15 X SeS-0.30).
[2] A,B - Differentiate selenium sources. a,b - Differentiate selenium levels.
* Dietary treatments that differ from the control diet by Dunnett's test (P<0.05).

Table 7 - Intestinal morphometry and mitosis rate in the duodenal epithelium of piglets fed diets enriched with proteinate selenium (SeP) or sodium selenite (SeS) at two addition levels (0.15 or 0.30 mg/kg)

	Duodenum				
	VH (μm)	VW (μm)	CD (μm)	VH:CD	Mitosis (n°)
Dietary treatment					
Control diet	293.15	135.47	96.93	3.60	767.615
SeP-0.15	273.46	120.98	101.06	2.98	752.802
SeP-0.30	251.03	101.98*	92.44	2.90	619.232*
SeS-0.15	298.10	116.08	97.41	2.94	909.812
SeS-0.30	261.96	101.57*	88.25	3.12	860.389
Dietary Se source					
SeP	262.24	111.48	96.75	2.94	686.017
SeS	280.03	108.83	92.83	3.03	885.101
Dietary Se level[1]					
0.15 (mg/kg)	285.78a	118.53a	99.23a	2.96	831.307a
0.30 (mg/kg)	256.49b	101.77b	90.34b	3.01	739.811b
ANOVA (P-value)					
Dietary Se source	0.070	0.526	0.985	0.530	<0.0001
Dietary Se level	0.001	0.001	<0.0001	0.769	0.031
Interaction	0.480	0.675	0.240	0.403	0.314
Pooled SEM	10.08	4.91	1.99	0.10	30.843

VH - villus height; VW - villus width; CD - crypt depth; VH:CD - villus height:crypt depth ratio; SEM - standard error of mean.
[1] a,b - Differentiate selenium levels.
* Dietary treatments that differ from the control diet by Dunnett's test (P<0.05).

There was an interaction effect (P = 0.027) for jejunal VW, where only diets with 0.30 mg/kg organic selenium reduced VW compared with 0.15 mg/kg. Compared with the control diet, both selenium sources at 0.30 mg/kg reduced VW. Crypt depth was higher in diets that added a selenium proteinate source over a sodium selenite source (P = 0.018), and in diets that added 0.30 mg/kg compared to 0.15 mg/kg (P = 0.024; Table 8).

Table 8 - Intestinal morphometry and mitosis rate in the jejunum epithelium of piglets fed diets enriched with proteinate selenium (SeP) or sodium selenite (SeS) at two addition levels (0.15 or 0.30 mg/kg)

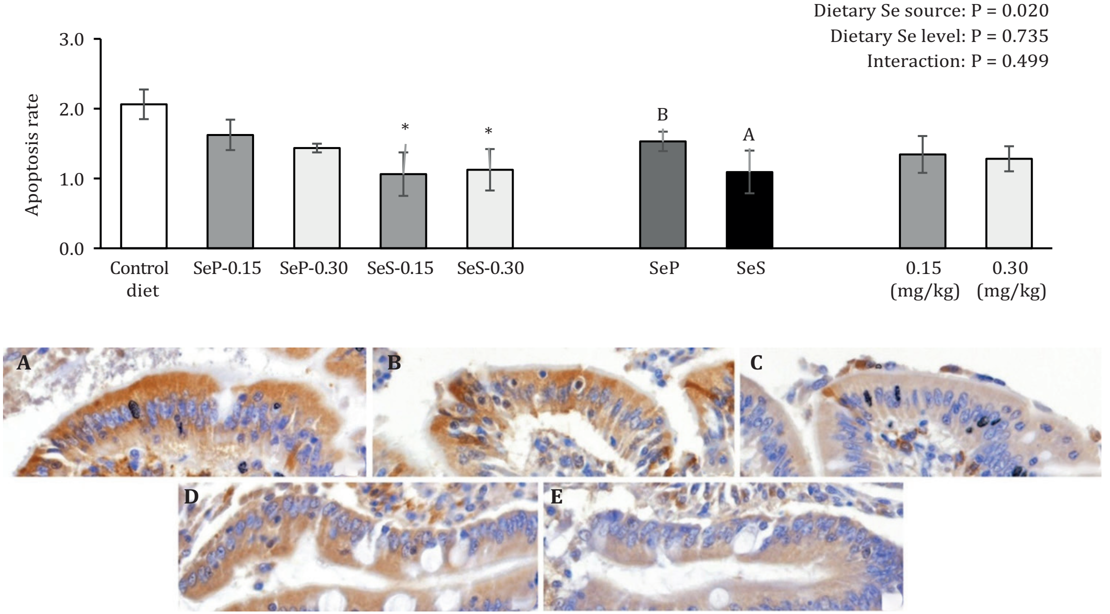
	Jejunum				
	VH (µm)	VW (µm)	CD (µm)	VH:CD	Mitosis (n°)
Dietary treatment[1]					
Control diet	218.69	107.60	87.97	2.68	872.645
SeP-0.15	236.08	108.24Aa	96.30	2.68	955.480
SeP-0.30	235.85	86.26Ab*	101.81	2.46	921.157
SeS-0.15	217.78	95.64Aa	86.03	2.70	997.790
SeS-0.30	229.33	90.10Aa*	96.67	2.51	916.568
Dietary Se source[2]					
SeP	235.97	97.25	99.06A	2.57	938.319
SeS	223.55	92.87	91.35B	2.60	957.179
Dietary Se level[2]					
0.15 (mg/kg)	226.93	101.94	91.17b	2.69	976.635
0.30 (mg/kg)	232.59	88.18	99.24a	2.49	918.862
ANOVA (P-value)					
Dietary Se source	0.123	0.237	0.018	0.674	0.585
Dietary Se level	0.564	<0.0001	0.024	0.083	0.097
Interaction	0.549	0.027	0.347	0.892	0.497
Pooled SEM	6.80	3.27	2.71	0.10	29.008

VH - villus height; VW - villus width; CD - crypt depth; VH:CD - villus height:crypt depth ratio; SEM - standard error of mean.
[1] A,B - Differentiate selenium sources at each level (SeP-0.15 X SeS-0.15 and SeP-0.30 X SeS-0.30). a,b - Differentiate selenium levels in each source (SeP-0.15 X SeP-0.30 and SeS-0.15 X SeS-0.30).
[2] A,B - Differentiate selenium sources. a,b - Differentiate selenium levels.
* Dietary treatments that differ from the control diet by Dunnett's test (P<0.05).

There was an effect on cellular apoptosis (P = 0.020; Figure 1). Lower cytoplasmic Caspase-3 positivity was observed in the jejunal epithelium of pigs fed selenium proteinate compared with inorganic selenium. When contrasted with the control diet, the diets with the inorganic selenium addition promoted a lower presence of cell apoptosis (P<0.05).

At 41 days of age, serum GGT concentration was lower (P = 0.051) in pigs fed 0.30 mg/kg selenium, regardless of source. Other parameters were not influenced (P>0.05) by the addition of selenium sources and levels, or even by concentration levels (Table 9). At 60 days of age (end of phase III), supplementation with 0.30 mg/kg selenium (regardless of source) increased serum CRC (P<0.0001) and IgA (P = 0.015) concentrations. The 0.15 mg/kg selenium proteinate diet resulted in lower CRC concentration (P<0.05) compared with the control, while the 0.15 mg/kg sodium selenite diet showed lower IgA concentration (P<0.05) compared with the control (Table 10). The malondialdehyde levels present in the muscle and jejunum of the pigs were not influenced (P>0.05) by the addition of selenium sources or levels.

Gene expression of GPX1 and GPX2 in the jejunal epithelium was not affected (P>0.05) by selenium supplementation at 0.15 or 0.30 mg/kg (Figure 2). Hepatic GPX1 expression was significantly higher (P<0.05) in pigs fed 0.30 mg/kg sodium selenite compared with the control diet (Figure 3). Hepatic SePP expression was significantly higher (P<0.05) in diets with 0.15 or 0.30 mg/kg sodium selenite compared with the control diet. Selenium source influenced the SePP expression (P = 0.038), with sodium selenite promoting higher hepatic SePP expression than selenium proteinate. Hepatic GPX4 expression was not affected (P>0.05) by the experimental diets.



A - control diet; B - SeP-0.15; C - SeS-0.15; D - SeP-0.30; E - SeS-0.30.
A,B - Differentiate selenium sources.
* Dietary treatments that differ from the control diet by Dunnett's test (P<0.05).

Figure 1 - Apoptosis rate in the jejunal epithelium of piglets fed diets enriched with proteinate selenium (SeP) or sodium selenite (SeS) at two addition levels (0.15 or 0.30 mg/kg).

Table 9 - Serum biochemical parameters at 41 days of piglets fed diets enriched with proteinate selenium (SeP) or sodium selenite (SeS) at two addition levels (0.15 or 0.30 mg/kg)

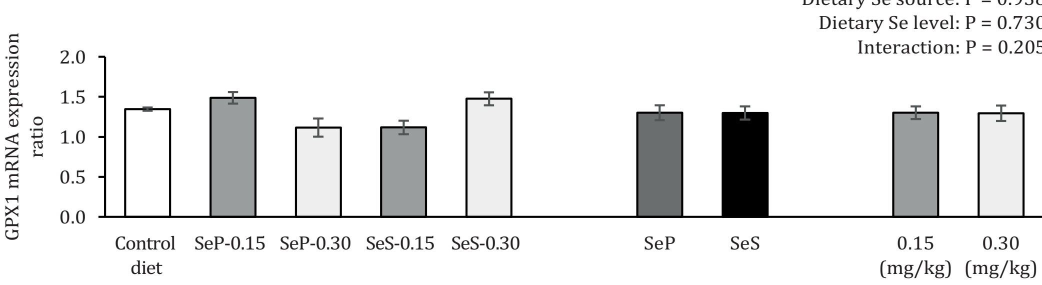
	41 days				
	GGT (U/dL)	AST (U/dL)	CRC (mg/dL)	IgG (mg/dL)	IgA (mg/dL)
Dietary treatment					
Control diet	8.11	9.17	2.15	488.3	47.73
SeP-0.15	8.51	9.68	1.85	463.2	43.63
SeP-0.30	7.83	9.52	2.20	483.4	46.93
SeS-0.15	8.37	9.51	2.45	479.9	45.03
SeS-0.30	7.46	8.54	2.50	487.7	45.63
Dietary Se source					
SeP	8.17	9.60	2.03	473.3	45.28
SeS	7.91	9.03	2.48	483.8	45.33
Dietary Se level[1]					
0.15 (mg/kg)	8.44a	9.59	2.15	471.5	44.33
0.30 (mg/kg)	7.64b	9.03	2.35	485.6	46.28
ANOVA (P-value)					
Dietary Se source	0.489	0.352	0.603	0.468	0.987
Dietary Se level	0.051	0.356	0.256	0.336	0.517
Interaction	0.748	0.505	0.695	0.664	0.651
Pooled SEM	1.689	2.235	0.154	5.245	1.035

GGT - gamma-glutamyltransferase; AST - aspartate aminotransferase; CRC - creatinine; IgA - immunoglobulin A; IgG - immunoglobulin G; SEM - standard error of mean.
[1] a,b - Differentiate selenium levels.

Table 10 - Serum biochemical parameters at 60 days of piglets fed diets enriched with proteinate selenium (SeP) or sodium selenite (SeS) at two addition levels (0.15 or 0.30 mg/kg)

	60 days				
	GGT (U/dL)	AST (U/dL)	CRC (mg/dL)	IgG (mg/dL)	IgA (mg/dL)
Dietary treatment					
Control diet	8.66	10.13	2.73	503.5	51.10
SeP-0.15	8.96	10.21	2.43*	497.1	46.97
SeP-0.30	8.89	9.90	2.80	503.6	50.05
SeS-0.15	9.17	10.60	2.55	502.6	41.48*
SeS-0.30	8.69	9.65	2.85	503.1	50.40
Dietary Se source					
SeP	8.92	10.05	2.62	500.4	48.51
SeS	8.93	10.12	2.70	502.8	45.94
Dietary Se level[1]					
0.15 (mg/kg)	9.06	10.40	2.49b	499.9	44.22b
0.30 (mg/kg)	8.79	9.77	2.83a	503.3	50.23a
ANOVA (P-value)					
Dietary Se source	0.948	0.894	0.226	0.197	0.348
Dietary Se level	0.370	0.132	<0.0001	0.089	0.015
Interaction	0.438	0.463	0.697	0.180	0.278
Pooled SEM	1.045	1.690	0.045	0.793	1.185

GGT - gamma-glutamyltransferase; AST - aspartate aminotransferase; CRC - creatinine; IgA - immunoglobulin A; IgG - immunoglobulin G; SEM - standard error of mean.
[1] a,b - Differentiate selenium levels.



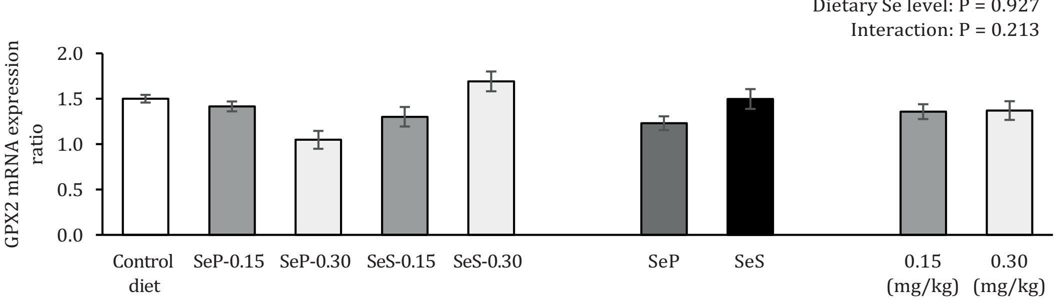
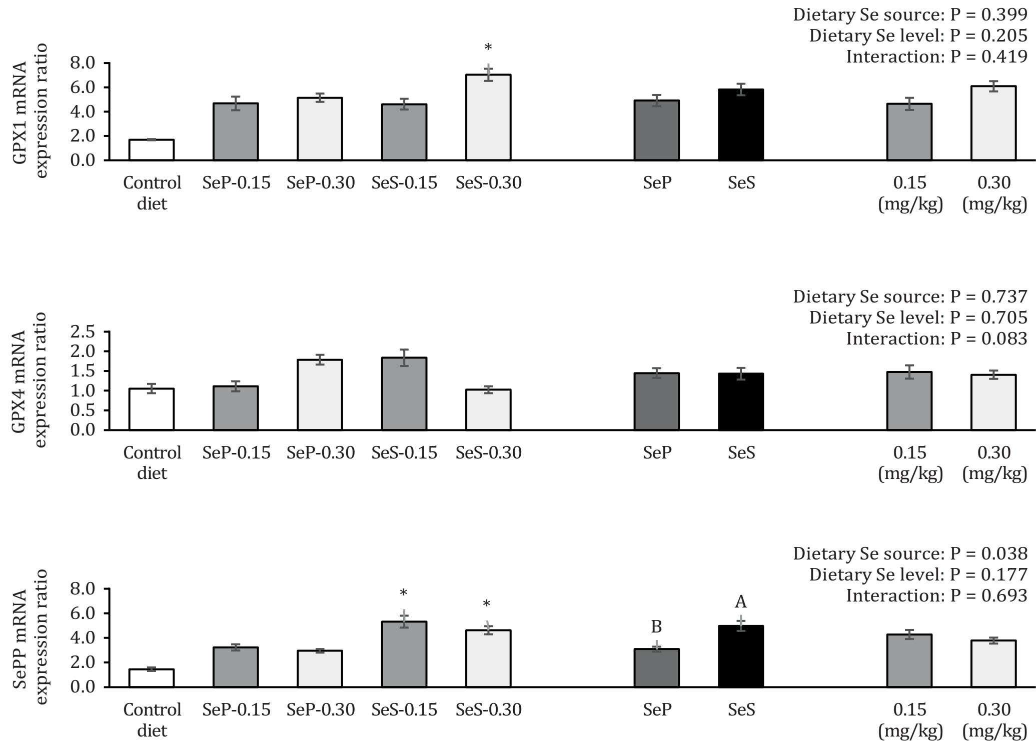


Figure 2 - mRNA expression of glutathione peroxidase 1 (GPX1) and glutathione peroxidase 2 (GPX2) in the jejunal epithelium of piglets fed diets enriched with proteinate selenium (SeP) or sodium selenite (SeS) at two addition levels (0.15 or 0.30 mg/kg).



A,B - Differentiate selenium sources.
* Dietary treatments that differ from the control diet by Dunnett's test (P<0.05).

Figure 3 - mRNA expression of glutathione peroxidase 1 (GPX1), glutathione peroxidase 4 (GPX4) and selenoprotein P (SePP) in the liver tissue of piglets fed diets enriched with proteinate selenium (SeP) or sodium selenite (SeS) at two addition levels (0.15 or 0.30 mg/kg).

4. Discussion

The highest selenium level (0.30 mg/kg) reduced ADFI and consequently ADG. Supplementation with 0.30 mg/kg selenium proteinate, in particular, reduced ADFI in both evaluated phases. A similar result was found by Rao et al. (2023) using selenomethionine the organic selenium source in piglet diets. Lyons et al. (2007) concluded that organic selenium sources are more bioavailable than inorganic ones, and therefore Rostagno et al. (2017) recommend 0.233 mg/kg selenium for newly weaned piglets when the source is organic, compared with 0.517 mg/kg for inorganic sources. In that study, the diets with the addition of 0.30 mg/kg of selenium had a selenium concentration of 0.576 mg/kg in their composition, resulting in values above the recommended level for organic sources (0.233 mg/kg), but close to that recommended for inorganic sources (0.517 mg/kg). The diets supplemented with organic selenium worsened the feed conversion of the animals from 28 to 60 days. This contrasts with Shen et al. (2009), who observed improved feed conversion with organic selenium supplementation, and Cao et al. (2014), who reported better feed conversion with selenomethionine-enriched diets.

Selenium proteinate improved dry matter digestibility in our study, which is similar to the results found by Shen et al. (2009) in evaluating the digestibility of pigs in the initial phase. They reported that a diet supplemented with selenium and yeast improved the dry matter, crude energy and crude protein digestibility. A reduction in feed intake by pigs fed a diet supplemented with 0.30 mg/kg of selenium may have influenced the digestibility of nutrients, as discussed by Li and Patience (2017). Changes in the intestinal microbiota may also have altered nutrient digestibility, since selenium is able to influence the microbiota as reported by Pereira et al. (2020). Nutrients not digested and absorbed in the small

intestine are fermented in the colon by the local microbiota (Wernimont et al., 2020), probably affecting the total apparent digestibility, which does not discriminate the contribution of microbial communities to nutrient digestibility.

The selenium concentrations in the diets with the addition of sources were higher than the recommendations of the NRC (2012) and by Rostagno et al. (2017) in seeking the effect on immune parameters and expression of selenoproteins related to antioxidant control. Therefore, there was greater selenium retention in all diets with selenium proteinate or sodium selenite added; on the other hand, diets with sodium selenite source addition and the diet with 0.30 mg/kg of selenium proteinate added provided greater selenium excretion than the control diet, indicating that there were other mechanisms in addition to the ADFI reduction to avoid toxicity due to excess selenium in the diet. Higher selenium retention resulted in greater muscle deposition, with tissue selenium concentration being higher with selenium proteinate than with sodium selenite, while the 30 mg/kg of selenium addition also increased the muscle concentration of selenium in relation to addition of 0.15 mg/kg. Our results are in line with those found in the literature. Rao et al. (2023) observed that an organic source of selenium resulted in higher selenium deposition in the tissue of piglets in the initial phase; Zhang et al. (2020) reported that sources and levels affect selenium concentrations deposited in the muscle, while Son et al. (2018) found that the selenium concentration level in the diet is directly correlated with concentrations in plasma, hair, liver, kidneys, muscles and urine of pigs.

Intestinal morphology is directly related to mitotic rate. Reduced villus height and width, as well as lower crypt depth in the duodenal epithelium of the pigs fed diets with 0.30 mg/kg selenium addition are related to the lower cell mitosis rate in the pigs with the same treatment. The reduced DFI observed in pigs fed 0.30 mg/kg selenium may have contributed that affected the morphology and cell mitosis in the epithelium of the pigs, since the intestinal segments need to obtain enough nutrients to meet the demands of mucosal protein synthesis and growth, causing shortening and modification in the villi structure and hyperplasia of the crypt cells (Pluske et al., 1997; Lin et al., 2014).

Supplementation with a sodium selenite source reduced the CD in the jejunal epithelium compared to selenium proteinate, whereas supplementation with 0.30 mg/kg of selenium reduced the villi width compared to the control diet. There is a relationship between DFI and intestinal morphology, similar to what occurred in the duodenal epithelium, however this response is also linked to the apoptosis rate, which was lower in the jejunal epithelium of pigs fed a diet supplemented with sodium selenite in relation to selenium proteinate and control diet. Gut morphology is linked to cell turnover (Pluske et al., 1997). Thus, apoptosis can influence crypt mitosis rate in the crypts, and consequently the values of villus height and crypt depth. For this reason, the highest selenium additional level can cause exaggerated inhibitory activity of oxidative processes, influencing the normal behavior of cell turnover in the intestinal epithelium.

Metabolic biomarkers are indicative of possible disturbances to the detriment of animal nutrition. AST and GGT enzymes are considered important markers of liver function and for evaluating the enzymatic metabolic profile, but damage to the liver tissue causes these enzymes to leak into the systemic circulation, thus altering their serum levels (Tennant and Center, 2008). It has already been reported in our study that the 0.30 mg/kg selenium addition in the diet resulted in DFI reduction mechanisms and increased selenium excretion, suggesting that this level may exceed the optimal threshold for piglets. CRC concentrations are considered normal for pigs between 1.0 and 2.7 U/dL (Kaneko et al., 2008). In this study, supplementation with 0.30 mg/kg selenium resulted in 2.83 U/dL, differing from the lower levels. Very high creatinine levels in the blood indicate impairment in kidney functionality, which may have been altered with the higher selenium level. The serum IgA concentration at 60 days was higher in pigs fed diets supplemented with 0.30 mg/kg of selenium, which is in line with the GGT and CRC results; however, the levels of antibodies found are below those suggested by Tizard (2019). In a study using selenomethionine at levels of 0.1 to 0.7 mg/kg of selenium, Cao et al. (2014) did not observe an effect on the IGA, IGG and IGM levels; nevertheless, the serum concentrations of these antibodies were higher than those found in our study.

The first selenoprotein identified in mammals was GPX1 (Rotruck et al., 1973). Together with GPX4, it is also the most abundant selenoproteins in various immune cells and tissues (Hoffmann et al., 2007; Carlson et al., 2010). GPX1 and GPX4 are expressed in most tissues, while GPX2 is mainly expressed in the epithelium of the gastrointestinal tract (Koyama et al., 1999). Although there was no difference in the GPX1 and GPX2 expression in the jejunal epithelium of the pigs in relation to the selenium addition sources or levels, GPX1 in the liver was more expressed when the pigs were fed a diet enriched with 0.30 mg/kg of sodium selenite compared to the control diet; this may have occurred due to GPX1 being more involved in antioxidant processes, being considered an essential selenoprotein. Furthermore, in the case of a change in selenium supplementation, its expression is prioritized to the detriment of other selenoproteins (Schomburg and Schweizer, 2009).

Hepatic SePP was directly influenced by the addition of sodium selenite in the diets compared to the addition of selenium proteinate and the control diet. SePP is synthesized in many different tissues, but it serves as a key transporter of selenium in the liver where hepatic SePP is secreted into plasma which does the transport, which in turn influences selenium homeostasis throughout the body (Schomburg and Schweizer, 2009). In our study, it was observed that the addition of sodium selenite resulted in greater selenium deposition in the muscle, as it is not complexed to an organic molecule; sodium selenite must be quickly transported to be excreted or deposited in the tissue, and so there is a greater expression of SePP to transport this selenium and maintain body homeostasis. There is also growing evidence that SePP not only transports selenium, but also plays crucial antioxidant roles, which are particularly important for certain immune functions (Huang et al., 2012).

5. Conclusions

At high concentrations, sodium selenite increases the expression of hepatic selenium-transporting selenoproteins, as well as serum creatinine and immunoglobulin A concentrations. Sodium selenite supplementation at 0.30 mg/kg may induce adverse effects, including altered intestinal morphology and kidney biomarkers. Selenium proteinate at 0.15 mg/kg appears to be a safer and more effective supplemental level, and its use is recommended as a dietary source of selenium at up to 0.15 mg/kg.

Data availability

The datasets generated during and/or analyzed during the current study are available from the corresponding author on reasonable request.

Author contributions

Conceptualization: Almeida, J. L. S. and Pascoal, L. A. F. **Data curation:** Almeida, J. L. S. **Formal analysis:** Almeida, J. L. S. **Funding acquisition:** Pascoal, L. A. F. and Guerra, R. R. **Investigation:** Almeida, J. L. S.; Almeida, J. M. S.; Batista, J. M. M.; Hermínio, M. P. F. and Araújo, W. J. **Methodology:** Almeida, J. L. S. and Pascoal, L. A. F. **Resources:** Watanabe, P. H. and Santos, V. L. **Supervision:** Pascoal, L. A. F. and Martin, T. D. D. **Writing – original draft:** Almeida, J. L. S. **Writing – review & editing:** Pascoal, L. A. F. and Givisiez, P. E. N.

Conflict of interest

The authors declare no conflict of interest.

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