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ANIMAL PRODUCTION

Dietary levels and sources of selenium for post weaning piglets

Tiago Ferreira Birro Oliveira[1] Antônio Gilberto Bertechini[1*] Renato Philomeno[1] Vanessa Avelar Silva[1]

[1]Departamento de Zootecnia, Universidade Federal de Lavras (UFLA), 37200-000, Lavras, MG, Brasil. E-mail: bertechini@dzo.ufla.br. *Corresponding author.

ABSTRACT: *An experiment was conducted to study the effects of two sources and dietary levels of selenium (Se) on performance, Se concentration and glutathione peroxidase activity in plasma and liver of piglets post weaning. A total of 126 weaned pigs male and female Large White × Landrace, at average body weight of 7.2±0.67kg were allotted to seven treatments in a randomized block design, with two Se sources (organic and inorganic), inclusion levels, two levels for the inorganic source (0.30 and 0.60ppm) and four for the organic source (0.15, 0.30, 0.45 and 0.60ppm) and one control diet without Se supplementation containing 0.095ppm, and six replicates of three animals per pen. The experimental period was 35 days post-weaning. The organic source contained 0.2067% (Se-yeast), and the inorganic 45.86% (sodium selenite) analyzed. Piglets that received supplemental Se had lower feed intake compared to those not supplemented. Furthermore, feed conversion was better with dietary supplementation of Se, and the organic source was better than the inorganic source at level of 0.30ppm. Plasma concentration of Se was higher (35.53%) in animals receiving dietary supplementation of this mineral, regardless of the sources studied. There was no effect of sources on plasma and hepatic Se, with the lowest values observed for the non-supplementation and organic Se at dietary level of 0.15ppm. There was a linear reduction of plasma GSH-Px activity with increased supplementation levels of organic Se, and the hepatic GSH-Px activity increased linearly with the increased supplementation, regardless of the source studied.*
Key words: *enzyme activity, glutathione peroxidase, hepatic selenium, plasmatic selenium.*

Níveis dietéticos e fontes de selênio para leitões pós-desmame

RESUMO: *Um experimento foi conduzido para estudar os efeitos de duas fontes e diferentes níveis de selênio (Se) sobre o desempenho, concentração de Se e atividade da glutationa peroxidase no plasma e fígado de leitões após o desmame. Um total de 126 leitões desmamados machos e fêmeas (Large White × Landrace), com peso corporal médio de 7,2±0,67kg foram distribuídos em sete tratamentos em delineamento em blocos casualizados, com duas fontes de Se (orgânico e inorgânico), com níveis de inclusão: dois níveis para a fonte inorgânica (0,30 e 0,60ppm), quatro para a fonte orgânica (0,15, 0,30, 0,45 e 0,60ppm) e uma dieta de controle sem suplemento de Se contendo 0,095ppm em seis repetições de três animais por baia. O período experimental foi de 35 dias após o desmame. A fonte orgânica continha 0,2067% (Se- Levedura) e a inorgânica (Selenito de sódio) 45,86% de Se analisado. Os leitões que receberam Se suplementar tiveram menor consumo de alimento em comparação com os que não foram suplementados. Além disso, a conversão alimentar foi melhor com a suplementação dietética de selênio e a fonte orgânica foi melhor do que a fonte inorgânica ao nível de 0,30ppm. A concentração plasmática de Se foi maior (35,53%) nos animais que receberam suplementação dietética deste mineral, independentemente das fontes estudadas. Não houve efeito de fontes no Se plasmático e hepático, com os valores mais baixos observados para a não suplementação e Se orgânico no nível de dieta de 0,15ppm. Houve uma redução linear da atividade GSH-Px plasmática com níveis de suplementação aumentados de Se orgânico e a atividade hepática GSH-Px aumentou linearmente com o aumento da suplementação, independente da fonte estudada.*
Palavras-chave: *atividade enzimática, glutationa peroxidase, selênio hepático, selênio plasmático.*

INTRODUCTION

The post weaning period is considered one of the most important phases in swine production, since piglets are exposed to stress factors that favor the development of pathogenic bacteria in the digestive tract, contributing to growth retardation and diarrhea during this period. In this regard, several measures can be taken to mitigate the adverse conditions of this phase, mainly in terms of well-being, thermal comfort, health and nutrition.

From a nutritional standpoint, adequate supplementation of organic selenium (Se), would be an alternative to minimize the problems during

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post weaning. Selenium has been show to have great importance for physiological functions and to improve performance (ZHAN et al., 2005) because it is indispensable in defending the body against free radicals causing oxidative damage of cell membranes (MAHAN et al., 1999; DOWNS et al., 2000; ZHAN et al., 2005). Stress by changing piglets diet and environment are important factors for the increase of free radicals. Moreover, the Se dietary sources studies, have shown that replacing inorganic by organic form, can increase both its absorption (MAHMOUD & EDENS, 2003), and its biological activity (WANG & XU, 2008; CAO et al., 2014).

The Se requirement for piglets has been established by NRC (2012) as 0.30ppm and ROSTAGNO et al. (2011) recommended 0.250ppm for all categories, from inorganic source only. However, ROSTAGNO et al. (2017) recommended for piglets post weaning 0.517ppm from inorganic source and 0.233ppm from organic sources.

The objective of this study was to evaluate the performance, plasma and hepatic Se concentrations, and activity of plasma and hepatic glutathione peroxidase (GSH-Px) of piglets in the post weaning phase with diets containing different dietary levels and sources of Se (organic and inorganic).

MATERIALS AND METHODS

One hundred and twenty six Large White x Landrace barrows and gilts weighing an average of 7.2±0.67kg were assigned in a randomized block design, according to body weight, even dietary treatments, six replications, and housed three per pen (2.0×1.2m) with individual semi-automatic feeders and drinkers, in a completely-enclosed, slotted-floor, environmentally-controlled building, during the 35 days of the experimental period (28 to 63 d of age). Water and diet was provided ad libitum during the whole experiment. The averages temperature and relative humidity of the room during the experimental period was 27.5±3.1ºC and 57.5%, respectively. Sodium selenite (45.86% of Se analyzed) as inorganic and selenium yeast (0.2067% of Se analyzed) as organic sources were used, respectively (Table 1).

Ingredient and nutrient specifications of the diets in all diets were identical, and the rations only differed in Se levels (Table 2). Corn-soybean meal basal diet formulation and nutritional composition shown in table 2 and the dietary nutrient components were according ROSTAGNO et al. (2011). Content of Se analyzed in the basal diet was 0.095ppm. Inorganic and organic Se sources was added using a premix with 1000ppm.

Table 1 - Experimental diets and specifications.

Diets	Specifications
1	Negative control without Se supplementation
2	With 0.30ppm of sodium selenite (45.86%)
3	With 0.60ppm of sodium selenite (45.86%)
4	With 0.15ppm of selenium yeast (0.2067 %)
5	With 0.30ppm of selenium yeast (0.2067 %)
6	With 0.45ppm of selenium yeast (0.2067 %)
7	With 0.60ppm of selenium yeast (0.2067 %)

Every day the stalls were cleaned and animals fed with dry feed at 7am and 4pm. At the end of the experiment an animal in each pen was slaughtered to collect blood and liver for the GSH-Px activity tests and selenium levels. The slaughters were according to Ministry of Agriculture Livestock and Food Supply (MAPA) standards. Blood collection proceeded after electric stunning of animals, during exsanguination. The blood collected in tubes containing anti-coagulant (sodium heparin) was centrifuged (Sigma 2-5) immediately at 3000x for 10 minutes. After completion of the process, the plasma portion was pipetted, placed in a graduated Eppendorf microtube (1.5ml) and frozen at -20°C for analysis of Se and the GSH-Px activity. Selenium analyses were conducted by atomic absorption spectrometry in a Spectra 2000 apparatus (Varian, Australia), equipped with 77 VGA system for hydride generation and 10mA cathode lamp (Varian, Melbourne, Australia). Digestion was carried out in a digester oven for simple digestion (Merck Darmstadt, Germany). The liver selenium content was performed on an L202 lyophilizer (Liobras), considering the initial weights and final samples. For the plasma samples preparation for reading the wet sample methodology was used. All quantitative analysis (digestion and reading) processes were performed by the same equipment mentioned above.

The readings of the GSH-Px were taken following the methodology described by LEVANDER et al. (1983) adapted by MOREIRA et al. (2001). Enzymatic activity readings were monitored by the change in spectrophotometer absorbance according to the oxidation of NADPH at 340nm, and the enzyme activity was expressed as mmol NADPH oxidized per minute.

Table 2 - Experimental diet composition.

Ingredient (%)	Control, ppm	---Inorganic Se, ppm---		---------- Organic Se, ppm ----------			
	0.00	0.30	0.60	0.15	0.30	0.45	0.60
Corn	22.725	22.725	22.725	22.725	22.725	22.725	22.725
Soybean meal,46%	20.000	20.000	20.000	20.000	20.000	20.000	20.000
Micronized soybean	15.000	15.000	15.000	15.000	15.000	15.000	15.000
Pre-gelatinized corn	23.000	23.000	23.000	23.000	23.000	23.000	23.000
Soy oil	1.000	1.000	1.000	1.000	1.000	1.000	1.000
Milk serum powder	15.000	15.000	15.000	15.000	15.000	15.000	15.000
Dicalcium posphate	1.380	1.380	1.380	1.380	1.380	1.380	1.380
Limestone	0.750	0.750	0.750	0.750	0.750	0.750	0.750
Salt	0.300	0.300	0.300	0.300	0.300	0.300	0.300
Vitamin premix[1]	0.100	0.100	0.100	0.100	0.100	0.100	0.100
Mineral premix[1]	0.100	0.100	0.100	0.100	0.100	0.100	0.100
L-lysine-HCl,78%	0.370	0.370	0.370	0.370	0.370	0.370	0.370
DL-methionine, 9%	0.080	0.080	0.080	0.080	0.080	0.080	0.080
L-threonine, 98,5%	0.012	0.012	0.012	0.012	0.012	0.012	0.012
Zinc bacitracin,15%	0.050	0.050	0.050	0.050	0.050	0.050	0.050
Colistin sulfate, 8%	0.025	0.025	0.025	0.025	0.025	0.025	0.025
Selenium yeast,2000ppm	0.000	0.000	0.000	0.015	0.030	0.045	0.060
Sodium selenite,45.6%	0.000	0.030	0.060	0.000	0.000	0.000	0.000
Inert (kaolin)	0.100	0.070	0.040	0.085	0.070	0.055	0.040
Total	100.00	100.00	100.00	100.00	100.00	100.00	100.00
----------Nutritional composition----------							
ME, kcal kg^{-3}	3421	3421	3421	3421	3421	3421	3421
CP, %[2]	20.43	20.43	20.43	20.43	20.43	20.43	20.43
Ca, %[2]	0.855	0.855	0.855	0.855	0.855	0.855	0.855
Total P, %[2]	0.629	0.629	0.629	0.629	0.629	0.629	0.629
Avail. P, %[3]	0.444	0.444	0.444	0.444	0.444	0.444	0.444
DM, %[2]	89.140	89.140	89.140	89.140	89.140	89.140	89.140
CF, %[2]	2.460	2.460	2.460	2.460	2.460	2.460	2.460
Total Lysine, %[3]	1.550	1.550	1.550	1.550	1.550	1.550	1.550
Digest. Lysine, %[3]	1.333	1.333	1.333	1.333	1.333	1.333	1.333
Digest. Methionine, %[3]	0.377	0.377	0.377	0.377	0.377	0.377	0.377
Digest. Threonine, %[3]	0.837	0.837	0.837	0.837	0.837	0.837	0.837
Digest. Tryptophan, %[3]	0.221	0.221	0.221	0.221	0.221	0.221	0.221
Se, %[4]	0.095	0.290	0.650	0.180	0.300	0.490	0.680

[1]Provided the following per kilogram of diet: Vit. A, 15.000IU; Vit. D_3, 2.000IU; Vit. E, 100IU; Vit. K_3, 3.3mg; Vit. B_{12}, 12.5µg; folacin, 2.5mg; phantotenic acid, 15.0mg; biotin, 0.2mg; niacin, 30.0mg; pyridoxine, 5.0mg; riboflavin, 6.5mg; thiamine, 3.0mg; antioxidant: 1.5mg; copper, 6mg; iron, 100mg; iodine, 0,14mg; manganese, 4mg; zinc, 100mg.
[2]AOAC Analysis of the Association of Official Analytical Chemists (2000).
[3]According to ROSTAGNO et al. (2011).

[4]Analyzed by atomic absorption spectrometry equipped with 77 VGA System for Generator Hydrides

Controls tubes were without substrate (blank enzyme) as comparison. The analyzed variables were weight gain (WG), feed intake (FI), feed conversion (FC), and plasma and liver contents and glutathione peroxidase activity according dietary levels and sources of selenium.

Data were submitted to analysis of variance using proc GLM of SAS - Statistical Software (2000). Orthogonal contrasts ($P<0.05$) were performed to compare the control average with the average of the other treatments supplemented with selenium, to compare mean levels of each

source and to compare the means among sources. Regression analysis was applied for dietary Se levels within each source.

RESULTS AND DISCUSSION

Dietary Se levels and sources had no effect on WG (P>0.05), on the other hand, the FI and FC were influenced (P<0.05) (Table 3). The piglets that received supplemental Se showed lower feed intake (FI) and better feed conversion (P<0.05), compared to those without supplementation. Comparing the NRC (2012) recommendation of supplementation there are no differences (P>0.05) in feed intake between the inorganic and organic forms of the mineral. Reduction in the FI of growing-finishing pigs was observed by KIM & MAHAN (2001); however, with higher dietary Se levels (>5ppm). These results cannot be physiologically explained.

It was observed that regardless of the Se level and source, the supplementation improved FC (P<0.05). Increase of Se supplementation in the organic source improved (linear effect) in the FC of the pigs (P<0.05). According to NRC (2012) recommendation, the organic source was better than inorganic to FC (P<0.05). On the other hand, no differences (P>0.05) were observed to FC between the supplementation of 0.15 and 0.30ppm of Se in the organic form. CAO et al. (2014) observed that Se supplementation improved the FC on the piglets regardless of source and the organic Se was not showed difference between levels (0.10 to 0.70ppm).

According to MOREIRA et al. (2001), the organic Se provides greater availability of biologically active Se in the blood and greater deposition of Se in the tissues. In fact, ZHAN et al. (2005) observed that the addition of organic Se to growing and finishing pigs increased the deposition of Se in the tissues, improved the antioxidant ability to protect the myoglobin and to preserve the integrity of the cell membrane. Thus, a possible explanation for the improved FC of piglets supplemented with the

Table 3 - Initial and final weight, daily feed intake, daily weight gain, and feed conversion of piglets according to sources and Se levels during 35 experimental days*.

Se suppl. Levels	----------Control----------	----------Inorganic----------		---------- Organic ----------			
(ppm)	0.000	0.300	0.600	0.150	0.300	0.450	0.600
Initial weight, kg	7.213	7.201	7.183	7.155	7.172	7.226	7.168
Final weight, kg	23.971	22.582	22.614	23.435	23.225	23.367	22.766
Weight Gain, kg	0.479	0.440	0.441	0.465	0.459	0.461	0.446
----------CV (%) = 9.9----------							
Feed Intake[1] , kg	0.770	0.690	0.670	0.710	0.690	0.680	0.650
Feed Intake,[2] ,kg	0.770a					0.682b	
Feed Intake,[3]		0.690a				0.690a	
Feed Intake,[4]		0.710a				0.690a	
----------CV (%) = 7.21----------							
Feed Conversion[5]	1.608	1.568	1.519	1.527	1.503	1.475	1.457
Feed Conversion[2]	1.608b					1.508a	
Feed Conversion[3]		1.568b				1.503a	
Feed Conversion[4]		1.527a				1.503a	
----------CV (%) = 4.21----------							

*Means followed by the same letter in the row do not differ from each other according to orthogonal contrasts.

[1]Linear effect (P<0.05) in the organic Se levels (Y=0.730 - 0.1267 X, r^2=0.96).

[2]Average of the control treatment compared to other treatments supplemented with Se (P<0.05)[3]Average organic source compared with inorganic Se source at the 0.30ppm level (P<0.05).

[4]Average of the 0.15ppm with 0.30ppm organic source (P>0.05).

[5]Linear effect (P < 0.05) in the organic Se levels (Y=1.550 - 0.1587 X, r^2 = 0.99).

organic source is that it can result in a more effective cellular response by providing a steady state delivery of Se response to a deficiency of Se in conditions where the body of the pig is challenged. Plasma and liver Se concentrations increased linearly (P<0.05) with supplementation increase (organic source), and higher (*P*<0.05) for animals receiving dietary supplementation of this mineral (Table 4), regardless of the sources studied. There was no effect (*P*>0.05) of the sources on the plasma and liver concentration of Se for the supplements of 0.30ppm; however, lower level to 0.15ppm compared with 0.30 ppm to organic form (*P*<0.05) was observed.

According to HERDT et al. (2000), the blood Se concentration is an important indicator of recent intake of Se by an animal. Likewise, according to VALK & HORSTRA (2000), the concentration of Se in the liver provides an accurate indication of intake of Se. YOON & McMILLAN (2006) and MAHAN et al. (2000), reported that the use of organic Se in the diets of mothers increased the concentration of Se in blood of piglets at birth. Results of our research, showed that supplementary Se over 0.300ppm do not alter their concentration in plasma, but the liver Se contents markedly increased as the dietary Se level increased, regardless of source.

As for the activity of plasma GSH-Px in (Table 5), it appears that piglets without supplementation of Se in their diet had higher activity of this enzyme in relation to those receiving inorganic or organic Se in the diet (P<0.05). This observation is contradictory for many authors and suggested that the content of selenium in the basal diet was sufficient to maintain the enzyme activity. CAO et al. (2014) observed that the highest serum and muscle GSH-Px activity was reported in treatment fed with 0.30ppm of organic Se compared to control treatment (low-Se) and the 0.30ppm sodium selenite groups. SUNDE (2001) noted that the enzyme activity was stabilized after 0.1ppm. The basal diet contained 0.092ppm in this research.

In addition, there was effect of Se sources on the activity of GSH-Px in plasma (*P*<0.05), when compared with the same dietary levels (0.300ppm). Animals supplemented with organic Se had, compared to those receiving the inorganic source, the lower GSH-Px plasma activity. When the activity of GSH-Px was compared within each source, we observed that as the level of supplementation increased, the GSH-Px plasma activity decreased (*P*<0.05) for inorganic (0.300 to 0.600ppm) and organic sources (0.15 to 0.45ppm).

Table 4 - Plasma and liver Se contents (ppm) of piglets according sources and Se levels during 35 experimental days.

Analysis	Control	----------Inorganic----------		----------Organic----------			
Se suppl. levels (ppm)	0.000	0.300	0.600	0.150	0.300	0.450	0.600
Plasma Se[1]	0.043	0.132	0.135	0.063	0.121	0.124	0.153
Plasma Se[2]	0.043b				0.121 a		
Plasma Se[3]		0.132a			0.121a		
Plasma Se[4]				0.063 b		0.121a	
----------CV (%) = 16.88----------							
Liver Se[5]	0.030	0.810	1.192	0.423	0.885	1.061	1.251
Liver Se[2]	0.030b				0.937a		
Liver Se[3]		0.810a			0.885a		
Liver Se[4]				0.423b		0.885a	
----------CV (%) = 17.12----------							

*Means followed by the same letter in the row do not differ from each other according to orthogonal contrasts.

[1]Linear effect (*P*<0.05) in the organic Se levels (Y=0.047 + 0.182 X, r^2 = 0.87).

[2]Average of the control treatment compared to other treatments supplemented with Se (*P*<0.05).

[3]Average organic source compared with inorganic Se source at the 0.30 ppm level (*P*<0.05).

[4]Average of the 0.15 ppm with 0.30 ppm organic source (*P*>0.05).

[5]Linear effect (*P*<0.05) in the organic Se levels (Y=0.24 + 1.773 X, r^2=0.94)

Table 5 - Plasma and hepatic activities of GSH-Px (µmol of NADPH/min) of piglets, according sources and Se levels during 35 experimental days.

Analysis	Control	----------Inorganic----------		----------Organic----------			
Se suppl. levels (ppm)	0.000	0.300	0.600	0.150	0.300	0.450	0.600
Plasma activities of GSH-Px	7.491	7.542	7.351	6.794	6.695	6.513	6.612
Plasma activities of GSH-Px[2]	7.491a				6.918b		
Plasma activities of GSH-Px[3]		7.542a			6.695b		
Plasma activities of GSH-Px[4]				6.794 a		6.695 b	
----------CV (%) = 0.62----------							
Hepatic activities of GSH-Px[1]	13.641	15.831	18.574	13.693	14.092	14.385	16.052
Hepatic activities of GSH-Px[2]	13.641 b				15.438a		
Hepatic activities of GSH-Px[3]		15.831a			14.092b		
Hepatic activities of GSH-Px[4]				13.693b		14.092a	
----------CV (%) = 1.06----------							

*Means followed by the same letter in the row do not differ from each other according to orthogonal contrasts.

[1]Linear effect ($P<0.05$) in the organic Se levels (Y=12.713 + 4.9133 X, $r^2 = 0.84$).

[2]Average of the control treatment compared to other treatments supplemented with Se ($P<0.05$).

[3]Average organic source compared with inorganic Se source at the 0.30 ppm level ($P<0.05$).

[4]Average of the 0.15 ppm with 0.30 ppm organic source ($P<0.05$).

There was a linear increase ($P<0.05$) in the hepatic GSH-Px activity as the dietary levels increased (Figure 1), independently of source. According to ACDA & CHAE (2002) there is a greater deposition of Se in tissues, when the organic source is used; however, the inorganic source increases the hepatic activity of GSH-Px. In fact, there was increased hepatic glutathione activity with increasing supplementation with inorganic selenium. Conversel, the increase of the organic source also resulted in increased activity of the enzyme in the liver of piglets.

Results of the present study showed that the recommendations of Se for piglets of 0.300ppm recommended by the NRC (2012) are suitable for both organic and inorganic sources; however, recent recommendations suggested a lower inclusion of organic Se for piglets in post-weaning 0.233ppm (ROSTAGNO et al., 2017).

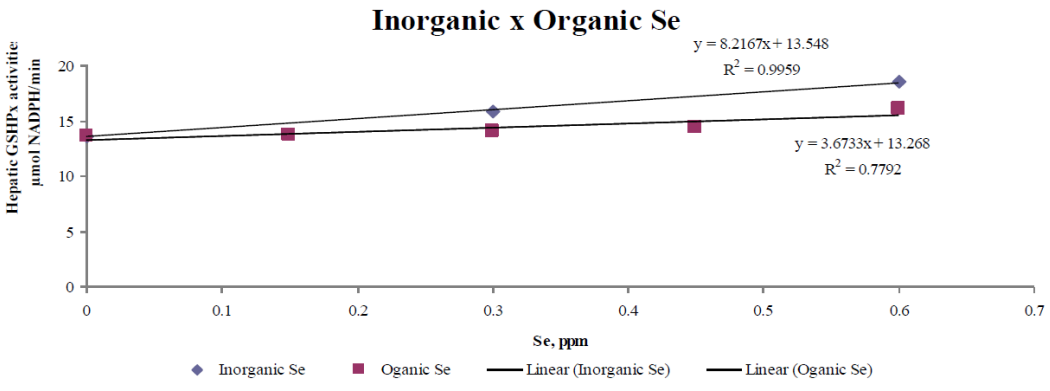

Figure 1 - Hepatic activity of GSH-Px according to Se levels.

CONCLUSION

The dietary supplementation of selenium improved the feed conversion without influence on the weight gain of piglets.

The feed conversion was better for the organic selenium source.

The liver and plasma selenium concentration increase with the selenium supplementation, regardless the source (organic or inorganic).

The plasma activity of GSH-Px decreased with the increase of the supplementation, regardless of selenium source.

The hepatic activity of GSH-Px is increased with increasing selenium supplementation, regardless of the source studied.

This study confirmed the recommendation of 0.300ppm of Se as suitable for piglet diets regardless of source.

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BIOETHICS AND BIOSSECURITY COMMITTEE APPROVAL

This research was approved by the Bioethics Committee of Universidade Federal de Lavras (UFLA), Minas Gerais, Brazil, protocol number 006/2016.

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