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ANIMAL SCIENCE

# Effects of organic Selenium- and Chromium-Enriched Diets on performance, carcass characteristics, lipid profile and fat quality of finishing pigs in different weight ranges

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**Abstract:** An experiment was conducted to evaluate the fatty acid profile of subcutaneous fat from barrowS of same genetic lineage supplemented with organic chromium and selenium initiated in different weight ranges in the finishing phase using 24 carcasses. Three different diets were used that represent the time when supplementation starts: control - without the inclusion of organic Cr and Se; CrSe70 - control with 500 g ton<sup>-1</sup> of organic Cr and Se of 70 to 130 kg in body weight; and CrSe100 - control with inclusion of 500 g ton<sup>-1</sup> of organic Cr and Se from 100 kg to 130 kg body weight. Performance, carcass characteristics, and lipid profile were evaluated. The data were submitted to analysis of variance, and with significant differences (p<0.05), the means were compared using the Tukey test. From 70 to 100 kg, control and CrSe70 animals consumed less feed than CrSe100. From 100 kg body weight, it reduced the C20:5n3 and C24:1n9 acids and increased the activity of the  $\Delta$ -6 desaturase, elongase,  $\Delta$ -5 desaturase enzymes in the supplemented animals. The moment when supplementation starts of organic chromium and selenium does not improve the performance and carcass characteristics, does not change the fatty acid profile, and does not improve the quality of the fat.

**Key words:** Lard, minerals, fatty acids, atherogenic and thrombogenic index.

# INTRODUTION

Supplementation with micro minerals valuable in the metabolism of lipids and carbohydrates, such as chromium (Cr) and selenium (Se), has been the alternative in farm animals to improve performance, carcass quality and reduced fat deposition (Mahan et al. 1999, Mateo et al. 2007, Domínguez-Vara et al. 2009, Tian et al. 2015, Caramori Júnior et al. 2017).

The use of micro minerals has been an alternative to ractopamine, questioned by the medical community for alleged health risks, causing restrictions on the use of this additive in certain countries, thus being considered a matter of food safety (Centner et al. 2014, Silva et al. 2014).

In addition to the performance and carcass characteristics, some researchers have observed changes in the fatty acid profile of adipose tissue (Jackson et al. 2009, Martínez-Gómez et al. 2012), also showing the ability of these minerals to alter the quality of meat and fat. The change in the fatty acid profile is dependent on several factors, and the phase in which supplementation starts, sooner or later, seems to have an effect on the response of the performance and carcass variables (Boleman et al. 1995, Gebhardt et al. 2016) and, therefore, could also influence the fatty acid profile. In this context, the objective of this study was to evaluate the appropriate moment in the finishing phase to start for the supplementation of organic Cr and Se aiming at a better performance, carcass characteristics and fatty acid profile of subcutaneous fat.

# MATERIALS AND METHODS

All experimental procedures were previously approved by the Committee of Ethics in the Use of Animals of the Universidade Federal do Mato Grosso do Sul (process # 840/2017).

#### Experimental design and diets

A completely randomized design of three diets and eight replications was used. In each diet, eight commercial hybrid pigs were used, barrow of same genetic lineage, totaling 24 animals. The basal diet (Table I) was formulated to meet the nutritional needs described by Rostagno et al. (2011). The different diets were considered treatments that represent the time when supplementation starts, following the scheme: 1) control - basal diet without supplementation of organic Cr and Se from 70 to 130 kg; 2) CrSe70 basal diet supplemented with 500 g ton <sup>-1</sup> of organic Cr and Se from 70 to 130 kg; 3) CrSe100 - basal diet supplemented with 500 g ton<sup>-1</sup> of organic Cr and Se from 100 to 130 kg. The supplementation of Cr and Se in the experimental diets were obtained from a commercial product composed by Saccharomyces cerevisiae yeast enriched with Cr and Se, containing a minimum of 1600 mg kg<sup>-1</sup> of organic Chromium and 1200 mg kg<sup>-1</sup> of Selenium, dry brewer's yeast, Schizochitrium sp., Ascorbic acid, and Aspergillus niger.

#### Performance and housing characteristics

The animals were weighed individually at the beginning of the experimental period with approximately 70 kg (66.86 ± 7.77 kg), close to

Ingredients (%)	70 - 100 kg	100 - 130 kg
Corn (7.88)	43.829	50.757
Soybean meal (46%)	21.495	14.496
Sorghum	29.993	29.993
Meat and bone meal (41%)	2.999	2.999
Vitamin/mineral premix <sup>1</sup>	1.500	1.500
L-Lysine	0.140	0.200
L-Threonine	0.020	0.030
Kaolin or Cr + Se supplement	0.025	0.025
Calculated composition <sup>2</sup>		
Crude protein, %	17.42	14.80
Metabolizable energy (Kcal kg <sup>-1</sup> )	3.330	3.330
Calcium, %	0.480	0.460
Available Phosphorus, %	0.260	0.250
Sodium, %	0.160	0.140
Digestible Lysine, %	0.830	0.710
Digestible Methionine + Cystine, %	0.470	0.410
Digestible Threonine, %	0.570	0.490
Digestible Tryptophan, %	0.200	0.140
Digestible Valine, %	0.730	0.620

<sup>1</sup>Supplied in 1 kg of product, 70 - 100 kg: choline 37.5 g; vit. A 1,625,000 IU; vit. D<sub>3</sub> 400,000 IU; vit. E 7,500 IU; vit. K<sub>3</sub> 750 mg; vit. B<sub>1</sub> 550 mg; vit. B<sub>2</sub> 1.375 mg; vit. B<sub>6</sub> 500 mg; vit. B<sub>12</sub> 5,000 mg; niacin 5,000 mg; pantothenic acid 2,300 mg; folic acid 125 mg; biotin 7.5 mg; Fe 25 g; Cu 3,750 mg; Mn 12.5 g; Zn 31.25 g; I 250 mg; Se 75 mg. 100 - 130 kg: choline 100 g; vit. A 6,000,000 IU; vit. D<sub>3</sub> 1,000,000 IU; vit. E 12,000 IU; vit. K<sub>3</sub> 1.5 g; vit. B<sub>1</sub> 0.5 g; vit. B<sub>2</sub> 2.6 g; vit. B<sub>6</sub> 0.7 g; vit. B<sub>12</sub> 0.015 g; pantothenic acid 10 g; folic acid 0.2 g; biotin 0.05 g; nicotinic acid 22 g; Fe 100 g; Cu 10 g; Mn 30 g; Zn 100 g; I 1 g; Se 0.3 g; Co 0.2 g. <sup>2</sup>Based on the composition of ingredients by Rostagno et al. (2011).

reaching 100 kg (104.75  $\pm$  8.28 kg) and at the end of the experiment with around 130 kg (130.23  $\pm$  8.86 kg).

To determine the average consumption, before each weighing of the animals, the leftover feed was subtracted from the total quantity supplied and then divided by the number of animals in the stall. Mortality was checked daily and was used to correct feed conversion calculations.

The 24 animals were slaughtered after a 12 h fasting in a commercial slaughterhouse following the rules established by the Ministry of Agriculture, Livestock and Supply in Brazil (Brasil 1995) for the slaughter of pigs. After eviscerated, the percentage of lean meat, the depth of the *Longissimus dorsi* muscle, and the thickness of fat were estimated using prediction equations generated by the Hennessy System GP4 software, after scanning the carcass with an electronic probe. Subsequently, the carcass bonus index was calculated according to the standards of the processing plant.

#### Sample collection

Fragments of the subcutaneous fat were removed using a scalpel, the collection being standardized in the region of the first lumbar vertebra (L1) of the left half carcass. Immediately after collection, the samples were stored in cryovials and immersed in liquid nitrogen for transport and then stored in an ultra-freezer at -80 °C for conservation and subsequent laboratory analysis.

# Analysis of the fatty acid profile

The technique for lipid extraction and fatty acid methylation was adapted from Hara & Radin (1978). After thawing, 1 g of fat sample was added to test tubes. Then, an isopropanol/ hexane mixture was added to the test tube to extract the fatty acids from the samples. In the end, a mix of extracted and air-dried lipids was obtained. For the methylation reaction of these fatty acids, approximately 40 mg of the sample of the extracted lipid mixture was weighed, which was placed in a test tube, and subsequently, the solvents (methyl acetate, sodium methoxide -30% in methanol) were added and anhydrous oxalic acid solution. The separation and detection of fatty acids were carried out by means of gas chromatography, using a gasliquid chromatograph Shimadzu model GC-2010 with flame ionization detector (FID), with a *split*/ splitless injector, in capillary column of fused

silica 30 m long, 0.25 mm in diameter, BPX-70 (70% Cyanopropylpolysilphenylsiloxane).

The operating parameters were set at detector temperature: 250 °C and injector temperature: 250 °C. The initial temperature of the column was 80 °C (2 min), gradually rising to 140 °C to 10 °C min<sup>-1</sup> and then to 240 °C to 5 °C min<sup>-1</sup> and remaining at that temperature for 10 min. For carrier gas, helium with a 1.0 ml min<sup>-1</sup> column flow rate, synthetic air, and hydrogen as the detector gas and nitrogen as auxiliary gas, make-up. For injection, 1 µL was used. The identification and guantification of fatty acids were performed by means of retention time, comparison of retention time (tr), and co-injection of fatty acid methyl esters from samples and standards (FAME mix, 100 mg - 37 components). Quantification was expressed as a percentage of the total sample.

# Lipid quality indices

The quality of fatty acids in adipose tissue in terms of the incidence of coronary heart disease was assessed using the atherogenic (AI) and thrombogenic (TI) indexes proposed by Ulbricht & Southgate (1991), according to the equations: AI = C12:0 + (4 × C14:0) + C16:0 / ( $\omega$ -6) + ( $\omega$ -3) + MUFA and TI = C14:0 + C16:0 + C18:0 / (0.5 × C18:1) + (0.5 × MUFA) + (0.5 ×  $\omega$ -6) + (3 ×  $\omega$ -3) + ( $\omega$ -3: $\omega$ -6).

The calculation of the iodine value (IV) was based on the AOCS Method Cd 1c-85 cited by Jackson et al. (2009), as well as the unsaturation index (UI) and the average carbon chain size (CC), according to the equations: IV = (C16: 1 × 0.95) + (C18: 1 × 0.86) + (C18:2 × 1.73) + (C18:3 × 2.62) + (C20:1 × 0.79); UI = 1 × (% monoenoic) + 2 × (% dienoic) + 3 × (% trienoic) + 4 × (% tetraenoic) + 5 × (% pentaenoic) + 6 × (% hexaenoic) and CC =  $\Sigma$  (n × (% fatty acid) / 100, being the number of carbon atoms.

The estimated activity rates of the enzymes desaturase and elongase were calculated using

the methodology of Korniluk et al. (2008), according to the equations:  $\Delta$ -5-desaturase = C20:4n6 / (C20:3n6 + C20:4n6);  $\Delta$ -9-desaturase = (C14:1 + C16:1 + C18:1) / C14:0 + C14:1 + C16:0 + C16:1 + C18:0 + C18:1);  $\Sigma$ C14,  $\Delta$ -9-desaturase = (C14:1) / (C14:1 + C14:0);  $\Sigma$ C16,  $\Delta$ -9-desaturase = (C16:1) / C16:1 + C16:0);  $\Sigma$ C18,  $\Delta$ -9-desaturase = (C18:1) / (C18:1 + C18:0);  $\Delta$ -6-desaturase, elongase,  $\Delta$ -5desaturase = C18:3 / (C18:3 + C20:5) and Elongase = (C18:0 + C22:1n9) / (C16:0 + C18:0 + C18:1n9 + C20:1n9).

#### Statistical analysis

The data were subjected to a simple analysis of variance through the GLM procedure in the statistical software SAS<sup>®</sup> (Statistical Analysis System University Edition), and in cases of significant differences, the means were compared using the Tukey test with statistical significance of p<0.05. The data of variables that did not follow a normal distribution were transformed following the sequence, first log transformation, then square root and then hyperbolic, and tested again for the assumptions after each conversion. If the data of the variables still did not meet the premises, nonparametric analyzes were performed and tested by the Kruskal-Wallis test with a statistical significance of p < 0.05.

# **RESULTS AND DISCUSSION**

Supplementation with organic Cr and Se did not alter daily weight gain and feed conversion (Table II). In the period from 70 to 100 kg, animals that received organic supplementation of Cr and Se since the beginning of the experiment (CrSe70) consumed less feed (p<0.05) compared to the control group. In the period from 100 to 130 kg,

	Control	CrSe70	CrSe100	P-value	SEM
70 to 100 kg BW					
IW <sup>1</sup>	66.250	68.100	-	-	1.588
FW <sup>1</sup>	104.388	105.488	-	0.680	1.690
DWG <sup>1</sup>	1.073	1.050	-	0.689	0.022
FC <sup>2</sup>	2.982	2.889	-	0.331	0.061
FA <sup>3</sup>	3.170a	3.019b	-	0.038	0.041
100 to 130 kg BW					
IW <sup>1</sup>	102.650	105.488	106.125	-	1.690
FW <sup>1</sup>	126.988	130.975	132.750	0.378	1.809
DWG <sup>1</sup>	0.785	0.822	0.859	0.378	0.021
FC <sup>2</sup>	4.298	3.861	4.144	0.452	0.130
FA <sup>3</sup>	3.301ab	3.163b	3.493a	0.005	0.052
70 to 130 kg BW					
IW <sup>1</sup>	65.750	68.100	66.750	-	1.588
FW <sup>1</sup>	126.988	130.975	132.750	0.124	1.809
DWG <sup>1</sup>	0.919	0.944	0.992	0.113	0.014
FC <sup>2</sup>	3.472	3.278	3.416	0.258	0.047
FA <sup>3</sup>	3.183b	3.086b	3.370a	<0.001	0.030

Averages followed by the same letter, on the line, do not differ statistically from each other by the Tukey test at 5% probability. Initial weight of each phase was used as a covariate in the statistical analysis. *BW* body weight *IW* Initial Weight, *FW* Final Weight, *DWG* Daily Weight Gain, *FC* Feed Conversion, *FA* Feed/Animal <sup>1</sup>kg <sup>2</sup>kg.kg<sup>-1</sup> <sup>3</sup>kg.day<sup>-1</sup> *Control* animals without supplementation *CrSe70* animals supplemented from 70 to 130 kg body weight, *CrSe100* animals supplemented from 100 to 130 kg body weight. the animals that received supplementation at the beginning of the experiment also (CrSe70) consumed less feed (p<0.05) than the animals that received late supplementation (CrSe100), but both did not differ from the control.

Considering the entire experimental period, the control animals and the animals that received supplementation from the beginning diet consumed less feed (p<0.05) compared to those that received supplementation after 100 kg body weight.

It has been observed that the supplementation of organic chromium can improve the nutritional digestibility of nutrients from diet, which reduces the consumption of animals, due to greater availability and use of nutrients (Oliveira et al. 2007), which is in agreement with the result obtained in the period of 70 to 100 kg between supplemented and non-supplemented animals. However, in the next phase, starting at 100 kg, animals supplemented from the beginning (CrSe70) consumed less feed than animals supplemented later (CrSe100), showing that the effect of chromium in improving the use of nutrients is more significant in the period of greatest muscle deposition in the finishing phase, from 70 to 100 kg, decreasing muscle deposition after

that period with an increase in the deposition of adipose tissue (Pereira 2014), with an increase in feed consumption.

The supplementation of organic chromium and selenium in the finishing phase, regardless of the weight range did not change the carcass characteristics of the animals in any of the treatments (Table III). In contrast to the present study, other authors obtained positive results, increasing the depth of the *Longissimus dorsi* muscle, reducing the thickness of the bacon, however, the minerals chromium and selenium were used alone (Wolter et al. 1999, Lien et al. 2001). Work developed by Rodrigues (2016), there was also no significant difference as expected, considering that in the association, they could enhance its positive effects.

In the review of the effect of high selenium intake and the risk of diabetes carried out by Steinbrenner et al. (2010), selenium with its insulin-mimetic effect and its protective antioxidant capacity, has been associated with type 2 diabetes, hyperglycemia and dyslipidemia in cases of supplementation, exerting an adverse effect on insulin-regulated metabolic pathways causing a *redox paradox* in its own signaling, in which reactive oxygen species stimulated

Variables	Control	CrSe70	CrSe100	P- value	SEM
HCW <sup>1</sup>	92.500	90.788	94.831	0.100	1.298
BL <sup>2</sup>	17.250	14.750	16.400	0.403	0.987
MDLd <sup>2</sup>	69.025	72.850	69.550	0.465	1.199
LM <sup>3</sup>	58.428	60.595	59.083	0.250	0.591
LMQ <sup>1</sup>	53.896	54.971	56.108	0.540	0.825
BCI	108.483	110.160	109.804	0.582	0.609

**Table III.** Carcass characteristics of swine supplemented with organic chromium and selenium from 70 to 130 kg body weight.

Averages followed by the same letter, on the line, do not differ statistically from each other by the Tukey test at 5% probability. Initial weight at 70 kg was used as a covariate in statistical analysis. *HCW* Hot Carcass Weight, *BL* Backfat Length, *MDLd* Muscle depth Longissimus dorsi, *LM* Lean Meat, *LMQ* Lean Meat Quantity, *BCI* Bonus Carcass Index; <sup>1</sup>kg <sup>2</sup>mm <sup>3</sup>%; *Control* animals without supplementation *CrSe70* animals supplemented from 70 to 130 kg body weight, *CrSe100* animals supplemented from 100 to 130 kg body weight. by insulin subsequently act on the signaling cascade.

By associating chromium and selenium, in order to capture more glucose and thus, greater protein synthesis, through the potentiation and signaling of insulin receptors by chromium, and the aid to the immune and antioxidant system performed by selenium through various selenoproteins, and in the action of inactivating reactive oxygen species, there may be direct interference of the action of chromium, raising the question and possibly not recommending the associated use of the two elements.

Eleven saturated fatty acids (SFA), eight monounsaturated (MUFA), and nine polyunsaturated (PUFA) were detected (Table IV). The acids, palmitic (C16:0), stearic (C18:0), oleic (C18:1n9), and linoleic (C18:2n6) were the ones that had the highest concentrations regardless of when supplementation started in the finishing phase.

Organic Cr and Se supplementation had a significant effect (p<0.05) only on the concentration of behenic (C22:0), eicosapentaenoic (EPA or C20:5n3), and nervonic (C24:1n9) acids. When animals are supplemented later, from 100 to 130 kg, reduced the concentration of C22:0 (p<0.05), compared to animals supplemented earlier, from 70 kg to 130 kg, both not differing from the control diet. For the C24:1n9 and C20:5n3 fatty acids, starting supplementation later decreases when compared to control (p<0.05), but did not differ from animals supplemented from 70 to 100 kg.

From 100 kg of body weight (BW), pigs increase the deposition of fat in the carcass (Pereira 2014), the fat composition being mainly influenced by the fatty acid profile from the diet, which is deposited directly in the adipose tissue, reducing lipogenesis by the *de novo* system, as well as insulin-dependent action (Smith et al. 1996, Azain 2004).

Supplement animals with organic Cr and Se from 100 kg (CrSe100) decreased C20:5n3 concentration (p<0.05), and even with the increase in the estimated activity of  $\Delta$ -6desaturase, elongase and  $\Delta$ -5-desaturase enzymes associated, the precursor fatty acid (C18:3n3), it was probably diverted to the formation of membrane phospholipids as a structural component or used as an energy source through β-oxidation (Lin et al. 1993) since only a small part of  $\alpha$ -linolenic acid is converted to very long-chain PUFA, and there is a possible preference for deposition in visceral adipose tissue compared to subcutaneous adipose tissue (Cunnane & Anderson 1997). In addition, C20:5n3 fatty acid participates in the carbon recycling process for lipid synthesis through de novo synthesis, where it is more readily β-oxidized compared to other PUFA (Gavino & Gavino 1991, Cunnane & Yang 1995), and contrary results help us to demonstrate that selenium did not reduce the oxidative damage of longchain PUFA, possibly due to the fact that the accumulation of this micro ingredient is less efficient in intramuscular fat (Korniluk et al. 2008).

Starting supplementation from 100 kg (CrSe100) reduced the concentration C24:1n9 acid, despite the abundance of MUFA in basal diets. Because it is a product of the elongation of the monounsaturated carbon chain, and the synthesis of its precursor is catalyzed by the enzyme stearoyl-CoA desaturase (SCD1 or  $\Delta$ -9-desaturase) (Nakamura & Nara 2004), suggests that organic Cr and Se act in the regulation of the SCD1 enzyme. In addition, MUFA are more easily accessible through synthesis by the *de novo* system than from the diet, which in general are immediately hydrolyzed and then oxidized, and another factor would be the regulation of lipogenesis by the diet's MUFA (Ntambi &

FA	Control	CrSe70	CrSe100	P-value	SEM
C10:0	0.0709	0.0852	0.0762	0.0722	0.0026
C12:0	0.0721	0.0785	0.0759	0.3703	0.0018
C14:0	1.2236	1.2652	1.3027	0.2801	0.0199
C14:1	0.0198	0.0211	0.0216	0.6876	0.0008
C15:0	0.0543	0.0585	0.0623	0.9026	0.0041
C15:1	0.0166	0.0167	0.0153	0.8677	0.0012
C16:0	24.8794	24.6949	24.9196	0.8741	0.1808
C16:1	1.5617	1.7471	1.6557	0.2182	0.0430
C17:0	0.3976	0.3944	0.4009	0.9854	0.0223
C17:1	0.2971	0.3362	0.3026	0.4546	0.0134
C18:0	13.9528	13.1618	13.5540	0.2463	0.1905
C18:1n9c*	40.4961	40.5695	40.0681	0.7334	0.3896
C18:2n6c	10.3868	10.5851	11.6216	0.4073	0.3865
C18:3n6	0.0967	0.1042	0.1007	0.7324	0.0037
C18:3n3	0.3834	0.4066	0.4150	0.5525	0.0125
C20:0	0.2210	0.2106	0.2081	0.5971	0.0053
C20:1	0.7702	0.7514	0.7502 0.9092		0.0201
C21:0	0.4230	0.4205	0.4489	0.4399	0.0098
C20:3n6	0.0832	0.0852	0.0855	0.9321	0.0028
C20:4n6	0.1837	0.1936	0.1974	0.8118	0.0081
C20:3n3	0.0625	0.0610	0.0629	0.8120	0.0012
C22:0	0.0136ab	0.0142a	0.0108b	0.0234	0.0006
C22:1n9	0.0152	0.0189	0.0156	0.3477	0.0015
C20:5n3	0.0097a	0.0081ab	0.0063b	0.0452	0.0006
C22:2	0.0093	0.0084	0.0068	0.1284	0.0006
C24:0	0.0916	0.0947	0.0952	0.9007	0.0036
C24:1n9	0.0101a	0.0085ab	0.0057b	0.0132	0.0007
C22:6n3	0.0109	0.0148	0.0111	0.0985	0.0009

# Table IV. Lipid profile (%), of the subcutaneous adipose tissue of swine supplemented with organic chromium and selenium.

Averages followed by the same letter, on the line, do not differ statistically from each other by the Tukey test at 5% probability. \*Means followed by the same letter, for the fatty acid C18:1n9c does not differ statistically from each other the Kruskal-Wallis test at 5% probability. *FA* Fatty acids, *C10:0* Capric acid, *C12:0* Lauric acid, *C14:0* Myristic acid, *C14:1* Myristoleic acid, *C15:0* Pentadecylic acid, *C15:1* Pentadecenoic acid (*cis-*10), *C16:0* Palmitic acid, *C16:1* Palmitoleic acid, *C17:0* Margaric acid, *C17:1* Heptadecanoic acid, *C18:0* Stearic acid, *C18:1n9c* Oleic acid, *C18:2n6c* Linoleic acid, *C18:3n6* γ-Linolenic acid, *C18:3n3* α-Linolenic acid, *C20:0* Arachidic acid, *C20:1* Eicosenoic acid, *C21:0* Heneicosylic acid, *C20:3n6 cis-*8,11,14-Eicosatrienoic acid, *C20:4n6* Arachidonic acid, *C20:3n3 cis-*11,14,17-Eicosatrienoic acid, *C22:0* Behenic acid, *C22:6n3* Docosahexaenoic acid; *Control* animals without supplementation *CrSe70* animals supplemented from 70 to 130 kg body weight, *CrSe100* animals supplemented from 100 to 130 kg body weight. Miyazaki 2003, Ntambi et al. 2004, Flowers & Ntambi 2008).

SCD1. induced by diets rich in carbohydrates. cholesterol. and saturated fatty acids. is activated by the sterol regulatory element-binding protein 1 (SREBP-1c) and liver receptor X (LXR). in the synthesis of MUFA. The increase in MUFA synthesis is associated with the activation of the genes for the enzymes acetyl-CoA carboxylase (ACC) and fatty acid synthase (FAS) by SREBP-1c. The importance of SCD1 in regulating lipogenesis noted by Ntambi et al. (2002), where the absence of SCD1 expression activated the AMP-activated protein kinase mechanism (AMPK) and the β3adrenergic receptor (\$3-AR). By different routes, the increase in AMPK reduces lipogenesis and increases β-oxidation, and β3-AR increases thermogenesis and metabolic rate. Together the two mechanisms work by decreasing the synthesis of lipids, suggesting that the supplementation of organic Cr and Se acts by reducing the expression of SCD1, consequently reducing the synthesis of very long-chain MUFA (C > 22), as observed in the results of the present study.

The reduction in the concentration of C22:0 fatty acid in pigs supplemented late (CrSe100) indicates that there may be a more significant desaturation of fatty acids. Besides that, Ohno et al. (2010) observed an increase in the amount of C24:0-CoA (tetracosanoyl-CoA) due to the performance and preference of ELOVL1 elongase over C18:0-CoA (stearoyl-CoA) and C20:0-CoA (araquidoyl-CoA) as substrate. This response can not only affect the concentration of C22:0, according to the result of the animals supplemented late (CrSe100) diet, but also the level of C24:0 fatty acid, but not observed in the present study.

The groups of fatty acids and the proportion of polyunsaturated:saturated (PUFA:SFA), were not significantly altered (p<0.05) by the supplementation of organic chromium and selenium (Table V).

The  $\omega$ -6: $\omega$ -3 ratio and the size of the carbon chain increased in the animal in the group of animals that were supplemented from 100 kg (CrSe100) (p=0.0166) and (p=0.0288), respectively, for animals supplemented earlier, started at 70 kg, both did not differ from the control diet. The atherogenic index, thrombogenic index,

	Control	CrSe70	CrSe100	P-value	SEM
SFA	41.4026	40.4897	41.1563	0.3820	0.2717
UFA	54.4400	54.9913	55.4046	0.4006	0.2852
MUFA*	43.2044	43.4904	42.8334	0.6554	0.4004
PUFA	11.2356	11.5009	12.5712	0.4292	0.4178
PUFA:SFA	0.2720	0.2851	0.3066	0.4849	0.0113
ω-3	0.4641	0.4867	0.4929	0.6730	0.0140
ω-6	10.7612	11.0072	12.0723	0.4006	0.4045
ω-9*	40.5411	40.6181	40.0880	0.7464	0.3901

Table V. Fatty acid groups of swine supplemented with organic chromium and selenium.

Averages followed by the same letter, on the line, do not differ statistically from each other by the Tukey test at 5% probability. \*Averages followed by the same letter, for AGMI and ω-9, do not differ statistically from each other by the Kruskal Wallis Test at 5% probability. SFA Saturated fatty acids, *MUFA* Unsaturated fatty acids, *MUFA* Monounsaturated fatty acids, *PUFA* Polyunsaturated fatty acids, *PUFA*:SFA Polyunsaturated fatty acids:Monounsaturated fatty acids ratio, ω-3 Omega-3 fatty acids, ω-6 Omega-6 fatty acids, ω-9 Omega-9 fatty acids; *Control* animals without supplementation *CrSe70* animals supplemented from 70 to 130 kg body weight, *CrSe100* animals supplemented from 100 to 130 kg body weight. unsaturation index, iodine value, and estimated activity of  $\Delta$ -5-desaturase,  $\Delta$ -9-desaturase, and elongase enzymes were not changed (Table VI).

The increase in the size of the carbon chain of fatty acids in the fat of the supplemented animals later (CrSe100) compared to those earlier (CrSe70) in the finishing phase, corroborates with the increase in the added activity of the  $\Delta$ -6-desaturase, elongase,  $\Delta$ -5desaturase enzymes, which is also higher in the animals supplemented from 100 kg (Table VII), obtained opposite results using a corn and soybased diet, and this information suggests that the composition of the diet has an important interference with the result obtained since the basal diet used in the experiment was composed of corn, soy, sorghum, and meat and bone meal.

Supplementation with organic Cr and Se can alter the metabolism of PUFA  $\omega$ -6 and  $\omega$ -3, suggesting the action of selenium against oxidative damage, however, in some fatty acids, such as C20:5n3, the response was the opposite, possibly due to the action of the Se specific in particular fatty acids and tissues as noted by Korniluk et al. (2008), in which the subcutaneous adipose tissue, a place where a lower concentration of Se is observed, thus less performance.

In the three fatty acids in which the concentration was reduced, as they are verylong-chain fatty acids, there was probably some interference in the enzymes responsible for the elongation of the carbon chain. The regulation of elongases is sometimes on different metabolic pathways that, through various stimuli, alter different sets of fatty acids in the cell, the degree of unsaturation and the length of the chain being relevant in the role in the regulation of these metabolic events (Jakobsson et al. 2006).

The  $\omega$ -6:  $\omega$ -3 ratio decreased in animals supplemented from the beginning (CrSe70) (*p*<0.05) compared to animals that received this supplementation later and for a shorter period (CrSe100) that can give better quality of fat.

Much is said about the ideal daily consumption of the ratio  $\omega$ -6: $\omega$ -3, with values below 4:1 being recommended (Scollan et al. 2006), amount around 1.0 g of the total fatty acids  $\omega$ -3 per day (Lottenberg 2009).

Prevention of cardiovascular diseases has been associated with the consumption of the recommended of  $\omega$ -6: $\omega$ -3 ratio. Still, the European OPTLIP study only stressed the

	Control	CrSe70	CrSe100	P-value	SEM
AI	0.5489	0.5432	0.5456	0.9343	0.0061
TI*	1.6956	1.7272	1.8647	0.5813	0.0651
UI	56.1457	56.7596	58.0806	0.2774	0.4965
IV	66.6791	67.4647	68.9564	0.3225	0.6161
CC	16.6933ab	16.6164b	16.8114a	0.0288	0.0312
ω-6:ω-3	23.2036ab	22.5884b	24.3655a	0.0166	0.2703

**Table VI.** Atherogenic, thrombogenic and unsaturation indices, iodine value, carbon length chain, and ω-6:ω-3 ratio, of swine supplemented with organic chromium and selenium.

Averages followed by the same letter, on the line, do not differ statistically from each other by the Tukey test at 5% probability. \*Averages followed by the same letter, for TI, do not differ statistically from each other by the Kruskal Wallis Test at 5% probability. *AI* Atherogenic index, *TI* Thrombogenic index, *UI* Unsaturation index, *IV* Iodine value, *CC* Average carbon chain size, *ω*-6:*ω*-3 omega-6:omega-3 fatty acids ratio; *Control* animals without supplementation *CrSe70* animals supplemented from 70 to 130 kg body weight, *CrSe100* animals supplemented from 100 to 130 kg body weight. importance of  $\omega$ -3 eating, since, in an attempt to determine a  $\omega$ -6: $\omega$ -3 ratio optimal, it was found that maintaining this proportion in the diet would not be so important, managing only to reaffirm the benefits of  $\omega$ -3, especially longchain ones, since the conversion of  $\alpha$ -linolenic into PUFA  $\omega$ -3 long-chain varies with the concentration of linoleic acid and  $\alpha$ -linolenic acid in the diet, and that their level would influence the conversion and not the relative proportions (Stanley et al. 2007, Griffin 2008). However, the results of the present research show that the supplementation of organic chromium and selenium earlier (CrSe70), improved the fat quality, considering the  $\omega$ -6: $\omega$ -3 ratio, compared to animals supplemented from 100 kg (CrSe100).

The value of the atherogenic and thrombogenic indices were not altered with the supplementation of organic Cr and Se in both treatments, regardless of when supplementation starts. As supplementation was not able to modify the concentration of monounsaturated fatty acids,  $\omega$ -6 and  $\omega$ -3, it was expected that there would be no change in the quality of fat linked to cardiovascular diseases. Ulbricht & Southgate (1991), when proposing the use of the indexes instead of the PUFA:SFA ratio, evaluated several foods, and the pig fat obtained the rates of 0.69, for AI and 1.66, for TI. In our results, the AI has rates around 0.54, and the TI

ranged from 1.6656 to 1.8647, showing that it is a healthy product with values very close to the recommendations for diet in terms of total fat.

Cardiovascular diseases, among others related to the consumption of fats, have been quite present in the daily life of the population today. When high values are observed for promoting protective rates, we must consider the consumption not only of the ingredient in question but also the presence of it in the composition of the diet. Recent pork consumption data (Associação Brasileira de Proteína Animal - ABPA 2018; OECD-FAO 2018), show per capita world consumption around 12.8 kg and in Brazil varying from 11.2 to 14.7 kg per year, considered relatively low compared to other countries in Europe, and regarding AI and TI, we have different compositions lipids depending on the cut evaluated (Bragagnolo & Rodriguez-Amaya 2002, Untea et al. 2017), which influences the values of the indices and cannot fail to consider that the preparation of food most often uses vegetable oils that can positively contribute to the balance of fatty acids in the diet (Wood et al. 2004, Garbowska et al. 2015).

Organic chromium and selenium when added to the pig diet from 100 kg (CrSe100), the estimated index of the associated capacity of the enzymes  $\Delta$ -6-desaturase, elongase and  $\Delta$ -5desaturase increased (p<0.05) when compared

	Control	CrSe70	CrSe100	P-value	SEM
	Controt	Cr5e/U	CrSeloo	P-value	SEM
∆-5 desaturase	0.6861	0.6943	0.6945	0.7275	0.0047
∆-9 desaturase	0.5122	0.5198	0.5116	0.4901	0.0030
ΣC14, Δ9-desaturase	0.0160	0.0164	0.0162	0.9475	0.0005
ΣC16, ∆9-desaturase	0.0589	0.0660	0.0622	0.0961	0.0013
ΣC18, ∆9-desaturase	0.7436	0.7550	0.7468	0.4173	0.0035
∆-6D, elongase, ∆-5D	0.9755b	0.9809ab	0.9857a	0.0198	0.0016
Elongase	0.1839	0.1757	0.1806	0.3643	0.0023

Table VII. Estimated enzyme activity of swine supplemented with organic chromium and selenium.

Averages followed by the same letter, on the line, do not differ statistically from each other by the Tukey test at 5% probability. *Control* animals without supplementation *CrSe70* animals supplemented from 70 to 130 kg body weight, *CrSe100* animals supplemented from 100 to 130 kg body weight.

with the pigs that received the control diet, but this same enzymatic activity did not differ significantly to the results of the animals in the group that received a diet with addition of organic chromium and selenium since the beginning of the period (CrSe70) (Table VII).

The increase in the associated activity of these enzymes was also observed by Nakamura & Nara (2004). These enzymes are found in higher level in the liver, although it was expected that even in the subcutaneous adipose tissue, due to the increased activity of these enzymes, that it would increase the concentration of very longchain PUFA (C > 22) series n-3 and n-6, however, it was not observed in this study, since for this it is necessary the presence of essential fatty acids 18:2n6 and 18:3n3, coming exclusively from the diet, as mammals are not able to synthesize these precursors from acetyl-CoA, as they lack  $\Delta$ -15 desaturase and  $\Delta$ -12 desaturase enzymes.

This result may be related to the time of onset or weight range for Cr supplementation and organic Se, where the greatest effect would be expected in the same period more fat deposition. Another reason would be that the time of use would influence the response, a fact observed by Alencar et al. (2021), where it was observed that the greatest response was obtained in 51 days of using chromium, and from that point on there was a decrease in its effect.

The significant effect observed in a few fatty acids, although supplementation started earlier (CrSe70) may be related to the fact that there was no significant effect on most performance variables and carcass characteristics. It would be expected that with the reduction of fat, as observed in several studies (Grela et al. 1997, Xi et al. 2001, Lien et al. 2001), this could lead to changes in the fatty acid profile.

The lack of results for this characteristic is also observed in other studies (Zhang et al. 2011, Peres et al. 2014, Xu et al. 2017), and possibly, the difference among the studies was because of different chromium sources, management, feed, sex, category and other factors (Lindemann 2008).

## CONCLUSIONS

Supplementation of organic chromium and selenium started in different moment of weight ranges in finishing pigs does not improve performance, carcass characteristics, fatty acid profile, and fat quality.

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EVF JGCJ, GSSC and CK conceived and designed research. EVF JGCJ and CK conducted experiments. EVF, SASA, EVF, JGCJ and LHV contributed new reagents. EVF, SASA, LHV and LFC analytical tools. EVF, JGCJ and GSSC analyzed data. EVF wrote the first version of the manuscript. JGCJ, CK, GSSC, SASA, LHV and LFC revised and edited final version of the manuscript. All authors read and approved the manuscript.

