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ANIMAL SCIENCE

Effect of chromium yeast supplementation on lipid profile of swine fat

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Abstract: This study was conducted to evaluate the period of chromium yeast supplementation on lipid profile of backfat and *Longissimus lumborum* muscle of barrows. It was evaluated carcass samples from forty barrows, genetically similar. Pigs diets were supplemented with 0.4 mg kg^{-1} of chromium yeast in four periods (0, 38, 62 and 94 days before slaughter). The experimental design was completely randomized with four treatments, ten replicates, and each experimental unit consisting of one animal. Lipid profiles of backfat and *Longissimus lumborum* muscle were analyzed by gas chromatography. The increase in the period of chromium yeast use had a quadratic effect ($P<0.05$) for stearic and oleic fatty acids, and total saturated, monounsaturated and unsaturated fatty acids in backfat. DH-γ-linolenic and arachidonic fatty acids reduced when the period of chromium yeast use increased. In the meat, there was a quadratic effect ($P<0.05$) only in the γ-linoleic fatty acid. The use of chromium yeast for different periods influences the lipid profile of the backfat and the *Longissimus lumborum* muscle, with less effect in the meat.

Key words: Atherogenic index, fatty acids, *Longissimus lumborum*, thrombogenic index.

INTRODUCTION

Chromium is an essential trace mineral that plays a key role in the metabolism of glucose, protein, and fat in animal tissue. Thus, supplementation of chromium emerged as a way to change the metabolism of pigs and produce carcasses with higher amounts of meat and lower fat (Ohh & Lee 2005).

There is a variety of forms of available chromium, and all these forms have different bioavailabilities, hence differing in performance responses (Lindemann et al. 2008). The organic sources of chromium, such as chromium picolinate, chromium nicotinate, chromium-methionine, and chromium yeast, are considered ten times more bioavailable than inorganic sources (Shelton et al. 2003), and because of this, they are the most used for better results (Park et al. 2009).

Among the responses found for the supplementation of organic sources of chromium, researchers reported improved feed conversion, increased weight gain, decreased fat, increased muscle percentage (Jackson et al. 2009, Li et al. 2013, Peres et al. 2014, Xu et al. 2017), and higher digestibility of the diet (Park et al. 2009).

In addition to the changes in performance and carcass, the observation of possible changes in lipid profile of swine fat has increased, due to the effect of fatty acids on human health, and the great interest of consumers about their diet. Therefore, studies that allow the evaluation of

each fatty acid, its sums, and indexes that may indicate fat quality (Ulbricht & Southgate 1991, Scollan et al. 2006) are necessary.

Considering that the effect of chromium on the fat reduction of the animal carcass is related, among other factors, to the activity of enzymes that synthesize fatty acids (Wang et al. 2014, Sadeghi et al. 2015), it is expected that their supply will affect the lipid profile of the fat depots.

However, contradictory results have been observed with different sources of supplementation, sex, management, feed, category, among others (Lindemann et al. 2008, Caramori Júnior et al. 2017). Among these factors, attention has been given to the fact that the supplementation period may influence the animal response (Boleman et al. 1995, Sales & Jancik 2011, Gebhardt et al. 2016, Caramori Júnior et al. 2017), it also could have an effect on the deposition of fatty acids in swine carcass.

Therefore, this study was carried out to evaluate the period of chromium yeast supplementation on lipid profile and indicators of fat quality in the backfat and L*ongissimus lumborum* muscle of barrows.

MATERIALS AND METHODS

The study was conducted at the experimental farm of the UFMS, located in Terenos, Mato Grosso do Sul, Brazil. Research of animals was conducted according to the institutional committee on animal use (UFMS 625/2014). It was evaluated forty carcass samples from barrows, genetically similar, that were supplemented with 0.4 mg kg^{-1} of chromium yeast in four periods (0, 38, 62, and 94 days before slaughter). The experimental design was completely randomized with four treatments, ten replications, and each experimental unit consisting of one animal.

Experimental diets (Table I) were elaborated based on corn and soybean meal to meet the nutritional requirements of barrows with high potential proposed by Rostagno et al. (2011). The chromium yeast was included in substitution for the inert material (kaolin). Mineral premixes used in the diets were devoid of chromium. The animals received the diets from 25 to 105 kg, and the experimental period was 94 days.

At the end of the finishing phase, the animals were fasted of solids for 8 hours. Afterward, they were transported to the slaughterhouse and they remained in rest pens for 4 hours. After this period, they were slaughtered according to the management standards and slaughter procedures of the Ministry of Agriculture, Livestock and Food Supply (MAPA) legislation. At the end of the slaughter line and before cooling carcasses, samples of the *Longissimus lumborum* muscle were removed with the subcutaneous fat at point P2 (region of insertion of the last thoracic vertebra with the first lumbar, six centimeters of the dorsal line). The samples were identified, packed, conditioned in a cooling box, and transported to the laboratory, where they were frozen for further analysis.

Lipid extraction and fatty acids methylation were adapted from Hara & Radin (1978). Samples were thawed and weighed 5 g of muscle tissue or 1.5 g of subcutaneous fat into a glass tube. To extract the fatty acids, 28 ml of hexane: isopropanol (3:2) mixture was added to the tubes, then homogenized with the samples and after standing, the mixture was filtered. For the removal of non-lipid substances, 12 ml of aqueous sodium sulfate was added to the filtrate, occurring separation in two phases. The upper part was separated containing hexane and lipids for further drying, obtaining at the end the extracted and dried lipids.

For the methylation reaction of these fatty acids, approximately 40 mg of this extracted

Table I. Composition of the experimental diets during the three phases.

	Phases[1]		
	25-50 kg	**50-75 kg**	**75-105 kg**
Ingredients (%)			
Corn	73.09	75.70	78.08
Soybean meal (45%)	23.41	21.43	19.30
Soybean oil	0.367	0.090	0.000
Dicalcium phosphate	1.181	0.953	0.869
Limestone	0.709	0.648	0.608
Vitamin/mineral premix[2]	0.400	0.400	0.400
Salt	0.405	0.379	0.354
L-lysine HCl	0.279	0.272	0.256
DL-methionine	0.074	0.059	0.048
L-threonine	0.063	0.053	0.056
Chromium yeast or kaolin	0.025	0.025	0.025
Calculated analysis			
Crude protein (%)	16.68	15.97	15.16
Metabolizable energy (kcal kg^{-1})	3,230	3,230	3,230
Lysine digestible (%)	0.943	0.891	0.829
Met+Cys digestible (%)	0.556	0.526	0.497
Threonine digestible (%)	0.613	0.579	0.555
Tryptophan digestible (%)	0.170	0.160	0.149
Calcium (%)	0.635	0.552	0.512
Digestible phosphorus (%)	0.314	0.269	0.250
Sodium (%)	0.180	0.170	0.160

[1]Duration of 32 (25-50kg), 24 (50-75kg), 38 days (75-105kg).
[2]Provided per kg of diet (25-75 kg): choline, 0.15 g; vitamin A, 6.500 UI; vitamin D3, 1.600 UI; vitamin E, 30 UI; vitamin K3, 3 mg; vitamin B1, 2.2 mg; vitamin B2, 5.5 mg; vitamin B6, 2 mg; vitamin B12, 20 mg; niacin, 20 mg; pantothenic acid, 9.2 mg; folic acid, 0.5 m; biotin, 0.03 mg; iron, 0.10 g; copper, 15 mg; manganese, 0.05 g; zinc, 0.125 g; iodine, 1 mg; selenium, 0.3 mg. Provided per kg of diet (75-105 kg): choline, 0.4 g; vitamin A, 24.000 UI; vitamin D3, 4.000 UI; vitamin E, 48 UI; vitamin K3, 6 mg; vitamin B1, 2 mg; vitamin B2 10.4 mg; vitamin B6, 2.8 mg; vitamin, B12, 0.06 mg; niacin, 88 mg; pantothenic acid, 40 mg; folic acid, 0.8 mg; biotin, 0.2 mg; iron, 0.4 mg; copper, 40 mg; manganese, 0.12 g; zinc, 0.4 g; iodine, 4 mg; selenium, 1.2 mg; cobalt, 0.8 mg.

fat was weighed, and placed in a test tube. The necessary solvents for the reaction (hexane, methyl acetate, 30% sodium methoxide in methanol) and anhydrous oxalic acid solution were added. After that, the esterified fatty acid samples were obtained for analysis by gas chromatography.

Fatty acids separation and identification were done using gas chromatography, using a Varian gas-liquid chromatograph CP-3800 with flame ionization detector (FID), with split/split less injector, in fused silica capillary column (30 m x 0.25 mm) with BPX-70 (70% Cyanopropyl polysilphenylenesiloxane).

The operating parameters were set at detector temperature: 250°C, and injector temperature: 200°C. The initial column temperature was 80°C (2 min), gradually increasing 4°C min^{-1}, until reaching 220°C (standing for 10 min). The carrier gas used was helium with a column flow of 1.0 mL min^{-1}, synthetic air and hydrogen were used as gas for the detector and nitrogen as auxiliary "make-up" gas. For injection, 1 µL of the solution was used. Identification and quantification of fatty acids were performed using retention time, comparison of the retention time (rt) and co-injection of fatty acid methyl esters from samples and standard (FAME mix, 100 mg - 37

components). Quantification was expressed as a percentage of total fatty acids identified and quantified.

The quality of the fatty acids profile was evaluated using two equations. Both were suggested by Ulbricht & Southgate (1991) for atherogenic index (AI) and thrombogenic index (TI): AI = (Lauric acid + (4 x Myristic acid) + Palmitic acid)/(Omega 6) + (Omega 3) + MUFA; TI = (Myristic acid + Palmitic acid + Stearic acid)/ (0.5 x Oleic acid) + (0.5 x MUFA) + (0.5 x Omega 6) + (3 x Omega 3) + (Omega 3/omega 6).

Desaturase and elongase enzymes were calculated according to Calvo et al. (2017): Delta-5 desaturase = (Araquidonic acid/ (DH-γ-linolenic + Araquidonic acid)); Delta-9 desaturase = ((Palmitoleic acid + Oleic acid)/ (Palmitic acid + Palmitoleic acid + Stearic acid + Oleic acid)); Elongase = ((Stearic acid + Gondoic acid[C20:1n9])/Palmitic acid + Stearic acid + Oleic acid + Gondoic acid)).

The data were submitted to analysis of variance by the GLM procedure of the SAS statistical program. Linear and quadratic regression analyses were also performed. The level of significance was 5%.

RESULTS

The period of chromium yeast supplementation influenced (P<0.05) the lipid profile of backfat and *Longissimus lumborum* (Table II). In the backfat (P<0.05), there was a quadratic effect (P<0.05) for stearic and oleic acid, with the highest effect found at 53 and 51 days, and linear reduction (P<0.05) for DH-γ-linolenic and arachidonic acid when the period of chromium yeast supplementation was increased.

In the *Longissimus lumborum*, there was a quadratic effect (P<0.05) of the γ-linolenic acid as a function of the chromium yeast supply period, with a minimum point of 53 days. The atherogenic and thrombogenic indexes, and the omega 6:omega 3 ratio for both tissues were not significantly different (P>0.05) for the different periods of use.

In the backfat, the increase of the chromium yeast supplementation period resulted in a quadratic effect (P<0.05) for saturated (SFA), unsaturated (UFA) and monounsaturated fatty acids (MUFA), with higher effect on 48, 48 and 58 days, without alteration (P>0.05) in the values of polyunsaturated fatty acids (PUFA). In the *Longissimus lumborum*, none of these variables were affected (P>0.05) by the period of supplementation.

The delta-9 desaturase and elongase enzymes in backfat were quadratically affected (P<0.05) as the supplementation period of chromium increased, with a greater effect within 50 days for both, whereas in the *Longissimus lumborum* there was no significant effect (P>0.05) for none of the enzymes evaluated (Table III).

DISCUSSION

Few studies in the literature evaluated the effect of chromium on the fatty acids profile of swine fat, and none of them evaluated the effect of

Table II. Fatty acid profile (% of total FA) of the backfat and *Longissimus lumborum* of barrows fed with chromium yeast for different periods before the slaughter.

	Supplementation period (days)				P-Value	CV (%)
	0	38	62	94		
Backfat						
Myristic (C14:0)	1.50	1.47	1.56	1.55	0.51	9.38
Palmitic (C16:0)	27.20	26.33	27.09	27.79	0.08	4.27
Palmitoleic (C16:1)	2.14	2.30	2.44	2.31	0.26	14.10
Stearic (C18:0)**	13.47	12.39	12.01	13.12	0.04	9.32
Oleic (C18:1n9)**	40.09	41.96	41.16	41.00	0.02	2.97
Linoleic (C18:2n6c)	11.60	11.69	11.79	10.33	0.18	13.77
γ-Linolenic (C18:3n6)	0.17	0.16	0.16	0.16	0.94	28.67
α-Linolenic (C18:3n3)	0.41	0.40	0.42	0.35	0.12	15.60
DH-γ-Linolenic (C20:3n6)*	0.07	0.07	0.07	0.05	0.02	19.23
Arachidonic (C20:4n6)*	0.17	0.16	0.17	0.13	0.02	21.58
Eicosatrienoic (C20:3n3)	0.06	0.06	0.06	0.06	0.19	12.29
Eicosapentaenoic (C20:5n3)	0.01	0.01	0.01	0.01	0.94	48.86
Docosahexaenoic (C22:6n3)	0.01	0.01	0.01	0.01	0.12	26.55
Saturated F.A.**	43.87	41.83	42.33	44.17	0.04	4.60
Unsaturated F.A.**	56.13	58.17	57.67	55.83	0.04	3.48
Monounsaturated F.A.**	43.57	45.58	44.96	44.70	0.02	2.98
Polyunsaturated F.A.	12.56	12.59	12.71	11.13	0.16	13.58
PUFA/SFA	0.29	0.30	0.30	0.25	0.15	17.67
Atherogenic index	0.59	0.56	0.58	0.61	0.08	7.56
Thrombogenic index	1.44	1.33	1.35	1.47	0.05	8.55
Omega 6:Omega 3	24.56	25.84	25.07	25.51	0.12	4.67
Longissimus lumborum						
Myristic (C14:0)	1.52	1.66	1.54	1.61	0.37	10.57
Palmitic (C16:0)	27.49	27.89	27.62	26.95	0.38	3.35
Palmitoleic (C16:1)	3.89	4.34	4.35	4.20	0.31	12.62
Stearic (C18:0)	12.42	11.72	11.30	11.36	0.06	6.70
Oleic (C18:1n9)	44.78	45.23	45.92	46.05	0.63	4.32
Linoleic (C18:2n6c)	5.69	5.19	5.49	5.51	0.93	28.23
γ-Linolenic (C18:3n6)**	0.14	0.11	0.12	0.14	0.03	18.95
α-Linolenic (C18:3n3)	0.15	0.15	0.16	0.16	0.91	20.29
DH-γ-Linolenic (C20:3n6)	0.15	0.12	0.12	0.13	0.78	46.26
Arachidonic (C20:4n6)	1.06	0.78	0.76	0.91	0.66	56.59
Eicosatrienoic (C20:3n3)	0.02	0.05	0.04	0.05	0.08	51.73
Eicosapentaenoic (C20:5n3)	0.01	0.01	0.01	0.02	0.49	57.61
Docosahexaenoic (C22:6n3)	0.02	0.02	0.02	0.02	0.97	58.81
Saturated F.A.	42.68	42.67	41.61	41.30	0.13	2.92
Unsaturated F.A.	57.32	57.33	58.39	58.70	0.13	2.13
Monounsaturated F.A.	49.90	50.78	51.55	51.60	0.49	4.36
Polyunsaturated F.A.	7.41	6.55	6.84	7.09	0.87	30.20
PUFA/SFA	0.17	0.15	0.16	0.17	0.87	30.66
Atherogenic index	0.59	0.61	0.58	0.57	0.43	5.90
Thrombogenic index	1.42	1.41	1.36	1.33	0.15	5.44
Omega 6:Omega 3	34.00	28.10	30.04	27.50	0.32	23.46

***Linear effect (P<0.05). Backfat: C20:3n6 = 0.0732 – 0.0002x; C20:4n6 = 0.1774 – 0.0004x. **Quadratic effect (P<0.05). Backfat: C18:0 = 13.506 – 0.0531x + 0.0005x²; C18:1n9c = 40.196 – 0.0516x + 0.0005x²; SFA = 43.843 – 0.0856 + 0.0009x²; UFA = 56.157 + 0.0856x – 0.0009x²; MUFA = 43.661 + 0.0585x – 0.0005x². *Longissimus lumborum*: C18:3n6 = 0.1416 – 0.0014x + 0.00001x².**

the supplementation period with any source of chromium. However, in some studies it was possible to observe changes in at least one fatty acid in some of the tissues evaluated (Grela et al. 1997, Lien et al. 2001, Jackson et al. 2009, Bučko et al. 2013, 2015, Untea et al. 2017), demonstrating the chromium use affects the fatty acids.

Chromium supplementation in different periods allowed the visualization of a quadratic response in the variables analyzed in the backfat (stearic acid, oleic acid, SFA, UFA, and MUFA) and meat (γ-linolenic). This effect indicates that there is a maximum result of the chromium in a certain period, and from that when it is prolonged its effect is reduced. In a meta-analysis (Sales & Jancik 2011), it was observed that the later the beginning of the chromium supplementation, the greater the fat reduction response and the increase of the meat deposition in the carcass, but did not indicate which was the most favorable period of use.

This was reported by Boleman et al. (1995), which supplemented pigs with 0.2 mg kg^{-1} of chromium picolinate, and obtained different responses for use in the growing and finishing phases (96 days) or only finishing phase (50 days), with a higher percentage of muscle for the last one. Another hypothesis would be that the effect would not be related to its use in a later period, but in a smaller period. Gebhardt et al. (2016) observed an increase in average daily gain and increase in feed intake up to the 39th day of use, and when they considered the whole period (117 days), they found differences only in daily feed intake.

The result for chromium is the same as that found to ractopamine use. To obtain the maximum response, it can not be used for more than 28 days, because after this period, there is desensitization of β-adrenergic receptors in cells (Moody et al. 2000). In chromium, this maximum response, where the greatest reduction or increase of fatty acids occurs, and possibly greater effect on performance and carcass characteristics, was observed around the 51 days of use before slaughter in this study.

In the literature, there are a variety of responses for the chromium use in lipid profile of swine fat. Some studies did not find effect on belly (Untea et al. 2017) or the loin (Jackson

Table III. Activity of enzymes in the backfat and *Longissimus lumborum* of barrows fed with chromium yeast for different periods before the slaughter.

	Supplementation period (days)				P-value	CV (%)
	0	38	62	94		
Backfat						
Delta-5 desaturase	0.70	0.70	0.71	0.70	0.98	6.97
Delta-9 desaturase**	0.51	0.53	0.53	0.51	0.02	3.36
Elongase**	0.17	0.16	0.16	0.17	0.02	7.43
Longissimus lumborum						
Delta-5 desaturase	0.87	0.87	0.86	0.87	0.67	2.20
Delta-9 desaturase	0.55	0.56	0.56	0.57	0.20	2.79
Elongase	0.15	0.14	0.14	0.14	0.10	6.95

**Quadraticeffect (P<0.05). Delta-9 desaturase = 0.5104 + 0.0009x – 0.000009^2; Elongase = 0.1734 – 0.0006x – 0.000006^2.

et al. 2009), while others observed effects on palmitic acid (Grela et al. 1997) linoleic acid (Grela et al. 1997, Lien et al. 2001), myristic acid and α-linolenic (Lien et al. 2001).

These variations in responses may be related to several factors such as dietary nutrient concentration, environment, management (Lindemann et al. 2008), sex, age, inclusion level and even the chromium source (Caramori Júnior et al. 2017) since even though organic sources have greater bioavailability than inorganic sources, there are differences between then.

As in the present study, other studies have also observed effect on MUFA (Bučko et al. 2013, 2015), SFA (Grela et al. 1997) and stearic and oleic acid (Lien et al. 2001). Considering that one of the main effects of chromium on pigs carcasses is the reduction of deposited fat, with consequent reduction of subcutaneous fat (Wang et al. 2007, 2014), this quantitative reduction of fat, may be accompanied by modification in the profile of fatty acids.

One expected effect of chromium is the increase in insulin sensitivity, leading to increased glucose uptake by the cells of the lean tissue (Lien et al. 2001), decreasing the plasma concentration (Wang et al. 2007, Peres et al. 2014, Xu et al. 2017), resulting in increased lean tissue and fat reduction. Increased insulin-like growth factor type 1 indicates this because it acts on protein metabolism and glucose uptake by muscle tissue (Wang et al. 2014).

Reduction of carcass fat occurs by reducing the plasma concentration of glucose, reducing the *de novo* synthesis, which is the process of fatty acids formation from other products and, consequently, the result can be expected is a reduction of SFA and MUFA, or specific fatty acids, as observed for stearic acid.

Other factors contribute to this conclusion such as the expression of genes related to enzymes involved in the processes of lipogenesis. Expression of the genes of the enzymes fatty acid synthase, diacylglycerol acyltransferase 1 and malate dehydrogenase were decreased with the chromium use (Wang et al. 2014, Sadeghi et al. 2015).

However, the reduction of the activity of these enzymes and *de novo* synthesis, contradict the results found for oleic acid and MUFA, since they should reduce following this logic. The result of chromium supplementation on the enzyme delta-9 desaturase would explain the response in these two variables.

The delta-9 desaturase enzyme is responsible for adding a double bond in stearic acid, forming the oleic acid. In the study of Sadeghi et al. (2015), the use of chromium had no significant effect on the expression of the gene related to this enzyme, but it was possible to observe that there was an upward expression in response to the increase in chromium level. Which could increase the oleic acid and, consequently, in MUFA and UFA.

About DH-γ-linolenic and arachidonic, there was a linear reduction as the period of chromium supplementation increased. These fatty acids can be derived from the diet or the synthesis from the linoleic acid substrate. The reduction of fatty acids indicates that chromium supplementation may have increased their use as a source of energy, decreased their deposition of even had effects on the delta-6 desaturase, elongase and delta-5 desaturase enzymes, which are necessary for formation of these fatty acids (Skiba et al. 2015).

The indirect evaluation performed in the present study there was no effect on the delta-5 desaturase, and the elongase enzyme was evaluated only on its effect on the increase in the carbon of the palmitic (C16:0) and oleic (C18:1) chains. However, a quadratic effect was observed, which could be extrapolated to the formation of DH-γ-linolenic acid, explaining

the reason for its reduction, and consequently that of arachidonic acid. If this same effect was observed for the enzyme delta-6 desaturase, it would explain the reason for the lower amount of γ-linolenic acid in the meat.

The differences for backfat and meat, when evaluating different diets, are commonly observed in several studies (Grela et al. 1997, Jackson et al. 2009, Alencar et al. 2017). This is mainly because meat fat has a lower presence of triglycerides due to its lower lipid deposition compared to subcutaneous fat and a higher amount of cell wall phospholipids that are less influenced by diet (Corino et al. 2014).

Although other studies indicate the effects of chromium supplementation on the lipid profile as in this study, changes in fatty acids were not enough to alter the indexes that indicate the quality of this fat for human health. The atherogenic and thrombogenic indexes that evaluate the fat capacity of food increase the chances of atherosclerosis and thrombosis (Ulbrich & Southgate 1991), respectively, did not change as the period of chromium yeast supplementation increased. As well as the omega 6: omega 3 ratio was not reduced to reach the levels considered adequate for human nutrition, which is 4:1 (Scollan et al. 2006), demonstrating the inefficiency of chromium yeast use to improve the quality of swine fat.

When comparing the inclusion of additives with the modification of dietary ingredients, it is possible to observe a greater effect of lipid sources on the lipid profile of swine fat, since it affects a greater amount of fatty acids, indexes, and their concentrations. These effects are observed for the inclusion of soybean oil (Alencar et al. 2017), linseed oil (Jiang et al. 2017), conjugated linoleic acid (Upadhaya et al. 2017), among others.

CONCLUSION

Increasing the period of chromium yeast supplementation before slaughter influence the lipid profile of the backfat and *Longissimus lumborum* muscle, with less effect on the meat.

Saturated, monounsaturated and polyunsaturated fatty acids are affected in backfat, whereas there is no effect on fat quality indexes in any of the evaluated tissues. The supplementation period with the greatest effect on fatty acids was around 51 days.

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