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EFFECT OF ORGANIC SOURCES OF CHROMIUM SUPPLEMENTATION WITH LOW LEVELS OF PROTEIN ON THE GROWTH PERFORMANCE AND RUMEN FERMENTATION PROFILE OF GROWING STEERS

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ABSTRACT

The aim of this study was to evaluate organic sources of chromium and the level of inclusion of these sources on the growth performance and rumen fermentation of Nellore Angus crossbred steers with low protein diets. Forty-eight crossbreed Red Angus Nellore, with body weight of approximately 200 ±14 kg and 10 months old, were used. Twelve animals were cannulated in the rumen to evaluate the dry matter degradability of Tifton-85 hay and ruminal fermentation profile. A randomized block design was applied, with a 3x4 factorial arrangement. Animals were distributed among the twelve diets, in which the three chromium sources were used. The chromium sources used were: 1- hrome sulfate, 2-chromium yeast, 3-chromium methionine, with four levels of inclusion 0, 1, 2 and 4 mg / animal / day. An effect for chromium sources and levels for dry matter intake was observed, as well as the effect of source and levels of inclusion of chromium and their interaction in the diets. For the concentrations of propionate, chromium source was important, with the highest percentage obtained for chromium yeast. Diets with low levels of crude protein are recommended for the growth of non-stressed crossbred Nellore Angus, with the additional use of chromium yeast or chromium methionine at the dose of 2 mg / animal / day.

Keywords: digestibility, chromium yeast, chromium methionine, ruminal fermentation, growth performance.

INTRODUCTION

Chromium is essential for the metabolism of carbohydrates and lipids (NRC, 2000) and, furthermore, is a structural component of "glucose tolerance factor," potentiating the action of insulin. Chromium supplementation in the diets of farm animal has been extensively studied in recent years, in order to improve performance, feed conversion, and promote changes in the carcass composition of these animals (Dominguez-Vara *et al.*, 2009).

Inorganic chromium is very poorly absorbed. Also, inorganic chromium must be converted to an organic complex, such as GTF, to enable the physiological functioning of chromium. Conversion of inorganic chromium (eg. chromium chloride) in the liver or kidneys to the bioactive form may be slow or even entirely nonexistent in certain individuals, particularly the aged ones (Swanson *et al.*, 2000).

Supplying chromium in the preformed complex organic form increases absorption, reduces variability in responses and negates the need for adequate dietary precursors (eg. nicotinic acid, certain amino acids) to aid inorganic chromium absorption and conversion to the bioactive form (Kegley and Spears, 1995).

Natural or synthetic organic chromium sources currently available in most human and certain animal markets include high chromium yeast, chromium picolinate, chromium nicotinate, chelated chromium and chromium proteinate (Swanson *et al* 2000).

High chromium yeast is usually more available for absorption (Mordenti *et al.*, 1997). Chromium yeast has been grown in the presence of high chromium concentrations. Yeast has the ability to accumulate ions, such as chromium, in high concentrations (Ingledew, 1999) and, therefore, can be useful as a source of supplemental minerals. Chromium methionine chelate is a newly developed organic mineral that is able to cross directly the intestinal cell membrane and be metabolized without any prior digestion, since it was chelated with amino acid. Therefore, bioavailability of chromium methionine chelate is proposed to be higher than those of other organic chromium (Ohh and Lee, 2004). The aim of this study was to evaluate organic sources of chromium and the levels of inclusion of these sources with low level of protein in the diet of to Nellore Angus crossbreed steers.

MATERIALS AND METHODS

The trial was conducted in Melon Institute, located in the city of Silvânia – GO, Brazil. Forty-eight crossbreed Red Angus Nellore were used with a live weight of 200 ±14 kg and aged 10 months. Twelve animals were cannulated in the rumen to conduct the dry matter degradability of Tifton-85 hay and ruminal fermentation. The design was block randomized in a factorial 3x4 (three sources and four levels) with three replications. The animals were distributed among the twelve experimental diets, where the three sources were: 1- chrome sulfate (Cr-S) ($Cr_2(SO_4)_3$); 2- chromium yeast (Cr-Y) (Co-Factor III, Alltech®), 3- chromium methionine (Cr-M) (Microplex, Zinpo®) and four levels of inclusion of each source @ 0, 1, 2 and 4 mg / animal / day.

The animals were allocated and initiated a period of 21 days corresponding to the adaptation on feed andpens. After this period, the animals were feedlot to experimental period of 84 days, with four periods of 21 days. The diets were balanced using the Cornell Net Carbohydrate and Protein System (CNCPS) (Fox *et al.*, 1992) and offered *ad libitum* twice daily, at 0700h and 1400h. The nutritional composition of experimental diets is presented in Table 1. Chromium was supplemented by four mineral mix (Table 2).

Performance, intake and digestibility: The animals were weighed at the beginning of the experiment and on the last day of each experimental period, always in the morning, after fasting for 12 hours of food and water.

Daily amounts of feed offered and orts, if any, were weighed and recorded for each steer. Diet samples of each steer were collected on d 15 to 18 for each period. Samples of all diet ingredients (0.5 kg) and orts (0.5 kg), from each animal were collected daily on d 15 to 18 and combined into one sample to represent 3-d digestibility determination. Samples of each individual ingredient were combined in the 3-d period. All samples were immediately frozen at −20°C.

For the determination of the flow of faecal dry matter was used chromium oxide (Cr_2O_3) as external marker, and 5.0 g in the morning and afternoon 5.0 g, orally, for 10 days of adaptation to treatment and 3 days of sample collection. The fecal samples were taken twice a day, from the rectum, according to the methodology described by Zinn *et al.* (1994).

The supplied ingredients and ort samples were analyzed for dry matter (DM), organic matter (OM), ash, fat, crude protein (CP) and lignin content according to the methodologies described by AOAC (2000). Crude protein (CP) content was determined by multiplying total nitrogen by 6.25. Non-fiber carbohydrate content (NFC) was estimated according to Hall (1998). Total digestible nutrients (TDN) were calculated according to Weiss *et al.* (1992). Neutral detergent fiber (NDF), and acid detergent fiber (ADF) contents were determined according to the method described by Van Soest and Mason (1991), using α-amylase without addition of sodium sulfite to determine NDF using an Ankom System.

Dry matter degradability: The disappearance of dry matter was based on the weight difference between the incubated material and the residues after incubation.

To estimate the kinetic parameters of DM, we used the first-order asymptotic model:

$$DP = a + b(1-e^{-ct})$$

Where:
DP=potential rumen degradability of feedstuffs;
a=soluble fraction;
b=potentially degradable fraction of the insoluble fraction that would be degraded at a rate c;
c=degradation rate of the fraction “b”;
t=incubation time in hours.
The fraction considered as undegradable calculated according to Orskov& McDonald (1979):

$$I = (100- (a+b))$$

The effective degradability (ED) is calculated with the following equation:

$$DE = a + [(b * c)/(c + k)]$$

Where k=passage rate of solids from the rumen, herein defined as 5.0% per hour (h), which can be attributed to the low, medium and high dietary intake.

Ruminal fermentation: Approximately 200 mL of ruminal fluid were collected at 2, 4, 6, 10 and 12 h after the morning feeding on day 19 of each treatment period. Then, pH values were determined using a potentiometer (Marte MB10, MG, Brazil). Aliquots of samples were mixed with 20% metaphosphoric acid (0.25 N HPO_3), centrifuged at 7000 × g, and stored at –20°C for subsequent analysis of short-chain fatty acids (SCFA). The remaining aliquots of samples were mixed with sulfuric acid (1 N H_2SO_4) and stored at –20°C for subsequent determination of ammoniacal nitrogen concentration (NH_3-N) according to the colorimetric method adapted by Foldager (1977).

Short-chain fatty acids (SCFA) were analyzed using the methodology proposed by Erwin *et al.* (1961), using a gas chromatograph (Model 9001 Gas Chromatograph, FINNIGAN BRAND) equipped with a glass column 2 meters in length × 0.25 m in width, packaged with 80/120 Carbopack B-DA/4% (Carbowax 20M, São Paulo, Brazil). The gases used in the analyses were nitrogen (25 mL/min flow) and hydrogen (15 mL/min flow) as carrier gases, and oxygen (175 mL/min flow) as oxidizer gas. The steamer temperature was set at 220º C, the ionization detector flames at 250º C, and the separation column at 195º C for 3 min, which was then raised 10º C/min up to 200ºC.

Statistical Analysis: Data were subjected to SAS (version 9.1.3, SAS Institute, Cary, NC 2004), verifying the normality of residuals and homogeneity of variances using PROC UNIVARIATE.

Data were analyzed by PROC MIXED according to the following model:

$$Y_{ijky} = \mu + B_i + A_j + S_y + L_k + S_y(L_k) + e_{ijyk}$$

In which: Y_{ijyk} = dependent variable, μ = general mean, A_i = animal effect, B_j= block effect, S_y = effect of chromium sources (i = 1 to 3), L_k = level of chromium effect (j = 1 to 4), $S_y(L_k)$ = interaction of chromium sources and levels and e_{ijk} = error. Random effect $A_j(B_i)$ = square into the block . The degrees of freedom were defined according to the method satterthwaite (ddfm=satterth).

To analyze diurnal patterns of ruminal fermentation characteristics (SCFA, NH_3-N) we added sampling time (0, 2, 4, 6, 8, 10or 12 h post-feeding) to the previous model and performed repeated measures analyses. Results of repeated measures analyses were subjected to three covariance structures: compound symmetric, first-order autoregressive, and unstructured. The covariance structure in each model that yielded the smallest Schwarz Bayesian criterion was considered the most desirable and reliable analysis.Data were subjected to analysis of polynomial regression by command PROC MIXED of SAS, version 9.0 (SAS, 2004), adopting a significance level of 5%.

RESULTS AND DISCUSSION

Performance: There was no effect (P>0.05) of the sources and levels of chromium for the variables dry matter intake (DMI% BW and% $BW^{0.75}$), initial body weight (BWI), final body weight (BWF) average daily weight gain (ADG), feed conversion (DMI / ADG) and feed efficiency (ADG / DMI).

There was observed effect (P<0.05) for chromium sources and levels for dry matter intake (DMI kg / day), were a quadratic response to the source of chromium yeast was observed ($Y = 5.30 + 0.51X - 0.31X^2$; P = 0.012; r = 0.65). The analysis of the regression equation revealed an optimal level of inclusion of high chromium yeast of 1.50 mg/animal/day. The dry matter intake estimated for chromium yeast was 4.47 kg / day.

The present study indicates that organic chromium and chromium yeast supplementations to growing non-stressed steers do not influence growth and gain efficiency. The data obtained for DMI, ADG, ADG / DMI and DMI / ADG are in accordance with the vast majority of studies found in the literature on the supplementation of beef cattle in the growth phase (Kegley *et al.* 2000, Swanson *et al.* 2000, Ohh and Lee, 2004)

Considering DMI, the results, obtained independently on chromium supplementation levels, may be related to the ability of chromium in its organic forms to module the glucose metabolism and subsequently regulate the concentrations of propionate inside the rumen. Chromium's primary function is to maintain glucose homeostasis, enhancing insulin action. In its physiologically active form, chromium decreases the amount of insulin required to maintain normal metabolism, acts as a cofactor and improves the efficiency of glucose uptake by cells (Allen, 1996).

Regarding the ADG, ADG/DMI, DMI/ADG, data in literature is varied. Chang and Mowat (1992) observed that weight gain of calves during the initial feedlot period was higher when animals received 0.4 mg of chromium yeast per kilogram of DMI. However, the final weight of the animals was not affected. According Mowat *et al.* (1993), the weight gain of calves supplemented with 0.5 mg of chromium yeast per kg DMI was not statistically higher than the control group when measured for a period of 35 days.

Likewise, daily supplementation per animal with 4.0 mg chromium yeast (Lindell *et al.*, 1994), 0.75 mg Chang *et al.* (1994), and 3.0 mg of chromium methionine (Mathison and Engstrom, 1995) did not significantly affect the performance of calves in the first 28 days of the experiment. The chromium supplementation in its organic form provides satisfactory results on the performance of feedlot cattle in the first twenty-eight days supplementation, due to adverse environmental conditions and the new social status of animals for longer periods such as this these studies beneficial effects are not observed in the majority of trials in the literature.

The quadratic effect observed for dietary chromium yeast can be linked to yeast's ability to more adequately stabilize the rumen fermentation events, thus enable the establishment of an appropriate level of inclusion of chromium in its organic form in growth diets for feedlot cattle. The estimated level of chromium yeast of 1.50 g/animal/day is in accordance with the NRC, (2000) recommendations for the category and breed animals used in this trial.

Apparent digestibility and dry matter degradability: Regarding the apparent dry matter digestibility, a significant effect (P<0.05) of source and levels of inclusion of chromium and interaction in the diets was observed. There was also a linear effect (P<0.05) for diet chromium yeast ($Y = 103.69 - 4.43X$; P = 0.014; r^2 = 0:50). According to the regression equation, for each increase of 1 mg/animal/day yeast chromium in the diet was reduced 4.43% *in vitro* digestibility of dry matter. A review of the chromium methionine diet presented a quadratic effect (P<0.05), where ($Y = 87.20 - 15.30X + 7.20X^2$; P = 0.024; r^2 = 0.63), and the optimal level of inclusion of chromium methionine was 1.06

mg/animal/day and the coefficient of digestibility of dry matter estimated for this inclusion level was 79.20%.

Source, level and interaction presented significant effects on the degradability of dry matter when the fraction a was analyzed. It was observed a quadratic effect (P<0.05) for chromium sulfate and chromium yeast diet, where ($Y = 17.72 - 7{,}69X + 1.39X^2$; P = 0.006; $r^2 = 0.53$), the optimal level of chromium sulfate inclusion was 2.76 mg/animal/day and the estimated value of the fraction a was 7.07. For chromium yeast ($Y = 11.67 – 3.87X + 1.06X^2$; P = 0.001; $r^2 = 0.46$) the optimal level of inclusion of was 1.82 mg/animal/day and the estimated value of the fraction a was 8.16. For the chromium methionine diet a linear effect was observed (P<0.05) (Y = 23.69 - 1.47X, P = 0.001, $r^2 = 0.47$), and for every increase of 1 mg/animal/day chromium methionine a decrease in the fraction A of 1.47 units was observed.

Regarding the fraction b, there was significant effect (P<0.05) of the inclusion level and interaction. It was observed a quadratic effect (P<0.05) for diet chromium yeast, with ($Y = 50.45 + 16.04X - 4.23X^2$; P = 0.021; $r^2 = 0.41$), 1.90 mg/animal/day being the best level of inclusion of chromium yeast and an estimated value for the fraction B of 65.66.

For effective degradability (ED) of dry matter, there were significant effects (P<0.05) of sources, levels and interactions. It was observed a quadratic effect (P<0.05) for chromium sulfate and chromium yeast diet, where ($Y = 30.42 – 4.13X + 0.85X^2$; P = 0.023; $r^2 = 0.62$), the optimal level of chromium sulfate was 2.43 mg/animal/day and the estimated value of ED was 31.93. For chromium yeast ($Y = 32.00 – 7.30X + 1.66X^2$; P = 0.010; $r^2 = 0.31$), the optimal level of inclusion of was 2.13 mg/animal/day and the estimated value of the ED was 24.01. For the chromium methionine diet, significant linear effect was observed (P<0.05) (Y = 46.60 - 1.80X, P = 0.018, $r^2 = 0.46$), and for every increase of 1 mg/animal/day chromium methionine, a decrease in ED of 1.80 units was observed.

The coefficients of apparent degradability of dry matter observed in this study are considered suitable for forage such as Tifton 85, which is characterized as highly digestible material through intrinsic conformation of its structural and non-structural components of the cell wall. Among the varieties of Cynodons, it is noticed that the Tifton 85 and 68 were more productive and had higher digestibility levels (Domínguez-Vara *et al.*, 2009).

The linear decrease in total apparent digestibility of dry matter observed for dietary chromium yeast may be associated with a negative interaction between source and level of chromium in the diet mainly 4 mg/animal/day of chromium yeast. Another factor that may be related to this result would be a possible toxicity of high levels of chromium yeast on rumen microbial population by reducing the synthesis of microbial protein and in consequence reducing the digestibility of dry matter.

The presence of L-methionine in chromium methionine diet may be related to the quadratic response observed, where the optimum addition level was 1.05 mg/ animal/day. Methionine may have contributed to microbial protein synthesis, thus providing the positive results observed in the digestibility of dry matter mainly for the diet with inclusion of 2 mg /animal/day of chromium methionine (Domínguez-Vara *et al.*, 2009).

Regarding the degradability of dry matter for the hay Tifton-85 in the fraction ‘a’ an average value of 9.24, regardless of the level and chromium source used, indicating that there was no loss of water-insoluble material. Reviewing degradability of dry matter of the fraction ‘a’ according to the experimental diets, average values of 8.97, 10.45 and 8.30 were obtained respectively for chromium sulfate, chromium yeast and chromium methionine. These values may be due to the low CP diets (9:20 %), or the high-level of chromium, 4 mg / animal / day.

The values of potentially degradable fraction ‘b’ of the dry matter of the roughage were 52.53, 56.31 and 55.71, respectively, for the diets with chromium sulfate, chromium yeast and chromium methionine, justifying the excellent quality of roughage used in this study. These data are also consistent with high dry matter digestibility in all the evaluated diets. The ED values of dry matter in this assay for the Tifton-85 were 27.70, 27.95 and 27.70 respectively for chromium sulfate, chromium yeast and chromium methionine, with an average of 27.78 experiment, indicating that the hay expressed its full potential degradation and digestibility in the rumen.

Rumen fermentation: Considering ruminal pH, a significant effect was observed for the sources and the level of chromium, and their interaction. The chromium sulfate diet presented a linear decreasing effect (P<0.05), with (Y = 19.7 - 0.14X, P = 0.001, $r^2 = 0.62$) and a reduction in pH of 0.14 units for each increase of 1 mg/animal/day. The chromium methionine diet presented quadratic effect (P<0.05), with ($Y = 6.94 + 0.03X - 0.01X^2$; P = 0.001; $r^2 = 0.63$), an optimal level of inclusion of chromium methionine of 1.50 mg/animal/day and an estimated pH value of 6.96.

Sources and levels of chromium, as their interaction, also presented a significant effect for N-NH3. There was a significant linear decreasing effect for the chromium sulfate diet, and for each 1 mg/animal/day, a decrease of 3.72 mg/dL N-NH3 in ruminal concentration was observed. The chromium yeast diet had a quadratic effect (P<0.05) with ($Y = 25.45 - 12.09X + 2.31X^2$; P = 0.001; $r^2 = 0.58$), best level of inclusion of chromium yeast of 2.62 mg/animal/day and an estimated concentration of N - NH3 of 9.63 mg / dL. A quadratic effect was also obtained for the chromium methionine

diet with ($Y = 27.47 - 11.91X + 2.12X^2$; $P = 0.001$; $r^2 = 0:49$), an optimal level of inclusion of chromium methionine of 2.80 mg/animal/day and an estimated concentration of N - NH3 of 7.95 mg / dL.

Only the source of chromium had a significant effect on propionate concentrations, with the highest percentage for chromium yeast diet (28.01%), compared to chromium sulfate (22.86%) and chromium methionine (22.97%).

For butyrate concentrations, an effect of sources, levels of chromium and their interaction was observed. The diet with chromium sulfate presented a quadratic effect with ($Y = 16.71 – 1.63X + 0.35X^2$; $P = 0.001$; $r^2 = 0.68$), the best level of inclusion of chromium sulfate of 2.32 mg/animal/day and the estimated concentration of butyrate of 14.82%. For the chromium yeast diet there was a linear decrease, with ($Y = 14.63 – 2.12X$, $P = 0.01$, $r^2 = 0:49$) and for each increase of 1 mg/animal/day was observed a decrease of 2.12 mg/dL in butyrate concentration. The chromium methionine diet presented a quadratic effect, with ($Y = 16.38 – 3.69X + 0.88X^2$; $P = 0.001$; $r^2 = 0:49$), the best level of inclusion of chromium methionine being 2.10 mg/animal/day and the estimated concentration of butyrate was 12.51%.

The pH values obtained from this trial were compatible with roughage and concentrations used .The linear reduction in pH presented by dietary chromium sulfate may be related to a possible toxicity level of 4 mg / animal / day strains of microorganisms lactate consumers, resulting in a small decrease in pH with increased levels of chromium sulfate. Regarding the chromium methionine diet the optimum inclusion was 1.50 mg / animal / day with an estimated value of 6.98, values that might be related to the presence of L-methionine in organic compounds, which provided better growing conditions of rumen microorganisms able to consume lactate, providing a more suitable pH (Kegley *et al.* 2000).

$N\text{-}NH_3$ concentrations in all the experimental diets may be associated with low crude protein level of the basal diet (9.20%). The linear decrease observed for dietary chromium sulfate may be related to the metabolism of inorganic chromium in reducing the rumen deamination of amino acids present in the diet. The diets chromium yeast and chromium methionine presented a peak of inclusion of organic chromium around 2.70 mg / animal / day. This level of organic chromium associated with this includes the effects of yeast and methionine complexes with chrome molecule. The level of inclusion of organic chromium is in accordance with (NRC, 1996, Kegley *et al.*, 2000, Swanson *et al.*, 2000, Ohh and Lee, 2004).

The chromium yeast diet presented higher concentrations compared to the others due to the fact that the presence of yeast provide stimulatory factors that increase the activity and the growth of ruminal bacteria. In this condition, there is an increase in ruminal degradation rates and fiber digestibility (Newbold *et al.*, 1996). The butyrate concentrations may be related to increased absorption of propionate in the rumen due to chromium supplementation, especially regarding the diet with chromium methionine. The greater absorption propionate provided higher concentrations of butyrate, whereas the great influence of chromium in glucose metabolism in growing ruminants. The diets chromium sulfate and chromium methionine presented good inclusion level of about 2.22 mg / animal / day, results that agree with most of the results presented in this study. The reduction in butyrate concentrations associated with dietary chromium yeast is related to the increase in propionate concentration previously offered by this diet (Ohh and Lee, 2004).

Table 1. Nutritional composition of the ingredients and experimental diet.

Nutrients	Tifton 85 Hay	Corn Meal	Mineral Mix	Diet
Dry matter, %	88.94	87.91	-	86.82
	% Drymatter			
Organic matter	92.20	97.54	-	92.12
Crude protein	9.69	9.05	-	9.29
Fat	1.55	4.02	-	2.33
Neutral detergente fiber	77.66	13.91	-	55.07
Acid detergente fiber	38.72	4.00	-	26.49
Non-fiber carbohydrate	8.26	71.55	-	28.98
Lignin	6.13	1.18	-	4.37
Ash	6.40	1.61	98.7	6.67
Total digestible nutrients	54.79	85.73	-	63.90

Table 2. Mineral composition of the mineral mix.

	ChromiumLevels			
Item	**0**	**1**	**2**	**4**
	Macrominerals (g/kg)			
P	81.0	81.0	81.0	81.0
Ca	105.0	105.0	105.0	105.0
Mg	5.0	5.0	5.0	5.0
N	20.0	20.0	20.0	20.0
Na	114.00	113.98	113.98	113.98
	Microminerals (mg/kg)			
Cr	**0.0**	**12.5**	**25.0**	**50.0**
Co	266.0	266.0	266.0	266.0
Cu	2204.0	2204.0	2204.0	2204.0
Fe	5000.0	5000.0	5000.0	5000.0
I	90.0	90.0	90.0	90.0
Mn	880.0	880.0	880.0	880.0
Mo	3.9	3.9	3.9	3.9
Ni	6.75	6.75	6.75	6.75
Se	9.0	9.0	9.0	9.0
Zn	6100.0	6100.0	6100.0	6100.0

Table 3. Performance according to the experimental diets.

Parameter[1]		DMI (kg/day)	DMI (%BW)	DMI (%BW$^{0.75}$)	BWI (kg)	BWF (kg)	ADG (g/day)	DMI/ ADG	ADG/ DMI
Cr sulfate	0	5.37	2.13	3.53	206.83	247.17	474	11.31	0.08
	1	5.48	2.24	3.58	197.83	240.83	506	10.98	0.08
	2	5.24	2.18	3.46	200.67	236.67	423	12.52	0.08
	4	5.44	2.29	3.56	194.83	235.17	474	11.86	0.09
Cr yeast	0	5.37	2.13	3.53	206.83	247.17	474	11.31	0.08
	1	5.79	2.34	3.73	197.50	241.83	521	11.21	0.09
	2	5.48	2.25	3.58	206.50	242.83	427	13.15	0.07
	4	4.66	2.22	3.17	169.25	207.75	453	10.81	0.10
Cr methionine	0	5.37	2.13	3.53	206.83	247.17	474	11.31	0.08
	1	5.56	2.36	3.62	192.00	230.00	447	12.61	0.09
	2	5.63	2.26	3.66	210.17	249.17	459	12.29	0.08
	4	5.40	2.24	3.54	198.08	239.58	488	11.08	0.09
SEM[2]		0.46	0.14	0.22	21.45	22.53	0.06	1.58	0.01
P value	Source	0.012	0.817	0.643	-	0.779	0.996	0.955	0.837
	Level	0.034	0.343	0.650	-	0.350	0.448	0.287	0.318
	Interaction	0.380	0.955	0.346	-	0.736	0.852	0.850	0.930

[1]DMI (Dry Matter Intake), BWI (Initial Body Weight), BWF (Final Body Weight), ADG(average daily gain), DMI/ADG(Feed Conversion); ADG/DMI(Feed Efficiency).[2]Standard error of mean.

Table 4. Digestibility and rumen degradation kinetics of dry matter of experimental diets.

Item[1]		Digestibility (%)	Dry Matter rumen degradation kinetics				
			a	b	c	DP	ED
Cr sulfate	0	79.40	10.96	52.25	0.03	63.22	30.37
	1	66.10	9.31	48.43	0.03	57.74	27.30
	2	68.80	5.83	58.33	0.02	64.16	25.48
	4	79.20	9.77	51.11	0.03	60.88	27.62
Cr yeast	0	79.40	10.96	52.25	0.03	63.22	30.37
	1	79.70	10.75	57.45	0.02	68.21	30.73
	2	74.30	6.73	69.21	0.01	75.94	20.78
	4	66.40	13.34	46.33	0.02	59.67	29.93
Cr methionine	0	79.40	10.96	52.25	0.03	63.22	30.37
	1	66.30	7.27	58.94	0.03	66.22	27.81
	2	60.00	9.67	51.65	0.02	61.33	28.40
	4	75.70	5.28	60.00	0.03	65.28	24.17
SEM[2]		0.10	2.58	8.00	0.01	7.02	3.43
P value	Source	0.014	0.008	0.248	0.537	0.157	0.941
	Level	0.025	0.001	0.050	0.273	0.384	0.001
	Interaction	0.013	0.001	0.046	0.613	0.157	0.001

[1]A (soluble fraction), B (potentially degradable insoluble fraction), C (degradation rate of potentially degradable fraction), DP (potential degradability), ED (effective degradability).[2]Standard error of mean.

Table 5. Ruminal fermentation according to experimental diet.

Item[1]			pH	N-NH_3 (mg/dL)	Acetate (%)	Propionate(%)	Butyrate (%)	Ac/Pr
Cr sulfate	0		7.02	25.93	59.73	23.56	16.70	2.53
	1		6.97	21.69	61.75	22.79	15.45	2.72
	2		6.78	17.72	61.52	23.67	14.80	2.60
	4		6.63	10.96	62.86	21.42	15.71	2.94
Cr yeast	0		7.02	25.93	59.73	23.56	16.70	2.53
	1		6.75	14.38	59.23	30.49	10.26	2.54
	2		7.11	11.47	59.05	31.30	9.63	1.89
	4		6.85	13.89	66.20	26.70	7.09	2.49
Cr methionine	0		7.02	25.93	59.73	23.56	16.70	2.53
	1		6.89	21.80	63.64	23.62	12.73	2.70
	2		6.49	9.04	64.95	21.86	13.18	2.97
	4		6.69	14.29	61.46	22.85	15.68	2.72
SEM[2]			0.23	7.91	6.28	6.99	3.78	0.42
P value		Source	0.013	0.007	0.599	0.001	0.001	0.341
		Level	0.001	0.001	0.149	0.446	0.001	0.134
		Interaction	0.001	0.001	0.194	0.334	0.001	0.562

[1]Ac/Pr (acetate / propionate ratio).[2]Standard error of mean.

Conclusion: According to the presented data, recommendation for diets with low levels of crude protein to growing crossbreed Nellore Angus is the additional use of chromium yeast or chromium methionine with an average dose of 2 mg / animal / day.

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