

Intake, digestibility and recovery of grains from feces of steers fed different oilseeds

Consumo, digestibilidade e recuperação de grãos nas fezes, de novilhos alimentados com diferentes grãos de oleaginosas

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ABSTRACT

Oilseed grains are used as a protein and energy source, increasing the energy density of the feed. The goal of the present study was to determine the nutritional profile of canola, crambe and soybean grains recovered from feces, and to evaluate the intake and digestibility of nutrients, rumen fermentation parameters of beef cattle receiving whole grains of oilseeds. Three steers, 350 ± 23.5 kg body weight were distributed in a not contemporary, 3×3 double Latin square, and fed oilseed grains. Inclusion of crambe grain reduced dry matter intake by 21.02%, with a higher digestibility coefficient for EE and NDF. The crude protein content of canola and crambe grains recovered were similar, but the EE content was higher for the crambe grain (26.46%). Soybean showed the lowest values of chemical composition for grains recovered from feces. The highest dry matter intake (7.86 kg/day), and the lowest NDF digestibility (0.203g/kg), were found for animals fed soybean. The inclusion of crambe grains reduced the rumen ammonia content (10.97 mg/dL); however, the ruminal pH was not altered by the inclusion of grains in the diet. Canola and soybean grains can be included in the cattle diet without altering dry matter intake;

while the inclusion of crambe grains reduces the dry matter intake and the ruminal ammonia content of the animals. Crambe grain showed the highest recovery of dry matter and ether extract from feces.

Key Words: Canola, Crambe, Digestibility, Recovery, Soybean.

RESUMO

Para se avaliar a inclusão de grãos inteiros das culturas de canola, crambe e soja na dieta de bovinos em confinamento, sobre o consumo, digestibilidade, parâmetros ruminais e recuperação dos grãos nas fezes, delineou-se um ensaio estatístico em quadrado latino com três dietas e três bovinos providos de cânula ruminal. Os resultados constataram que a inclusão do grão de crambe reduziu o consumo de matéria seca em 21,02%, e proporcionou aumento na digestibilidade do extrato etéreo e fibra em detergente neutra. O percentual de extrato etéreo recuperado nas fezes foi de 44,15% para grão de canola e 61,53% para o grão de crambe. Encontrou-se menor digestibilidade da fibra em detergente neutra para dietas contendo grão de soja 0,203g/kg, embora seu consumo aumentou em relação ao crambe 7,86 kg/dia. A inclusão de crambe apresentou redução nos teores de amônia ruminal (N-NH₃), com valores médios de 10,97 mg/dL. Portanto, o grão de crambe pode ser incluído na dieta de bovinos de corte em 14% da MS, sendo uma alternativa em potencial como grão de oleaginosa na alimentação de ruminantes. Assim como, os grãos de canola e soja sem afetar a digestibilidade da matéria seca e da proteína bruta, contudo, devem ser usados com cautela.

Palavras Chaves: Canola, Crambe, Digestibilidade, Recuperação, Soja

INTRODUCTION

Oilseed grains are used as a protein and energy source due to the high lipid content inside the seed, which is surrounded by a protein matrix, which can prevent the rapid release of lipid content in the rumen environment and reduce the negative effects on ruminal digestion of fiber (Rennó et al., 2015). Soybean grain is very widespread, and its use is possible both in the animal feed industry and in the human industry, therefore, there is competition for its use. It is an easy source to be purchased and its by-products are used more for animal feed. Canola grain is mainly used for oil production, as it is a rich source of unsaturated fatty acids, with functional potential, however, it has a higher added value. Crambe grain is a rich source of lipids and proteins; has 35% oil, which basically consists of 90.1% unsaturated

fatty acids, with 56.7% of this composition as erucic acid (Jasper et al., 2013), its main antinutritional factor, and should be added with caution in animal feed (Goes et al., 2016).

Several researches are carried out to evaluate the inclusion of oilseed grains in ruminant feeding, as a source of energy and protein for animal production, on the intake and digestibility of nutrients (Goes et al., 2011; Bassi et al., 2012; Rennó et al., 2015; Côrtes et al., 2015), however, Rennó et al., (2015) and Côrtes et al., (2015) highlighted that the chemical composition of grains recovered from feces is related to efficiency of nutrient use by animals.

The goal of this study was to determine the nutritional profile of canola, crambe and soybeans recovered from feces, as well as to evaluate nutrient intake and digestibility, ruminal fermentation

parameters of beef cattle receiving diets with inclusion of whole oilseed grains.

MATERIAL AND METHODS

The experiment was conducted in the Ruminant Nutrition sector, Animal Nutrition Laboratory (LANA), School of Agricultural Sciences, Federal University of Grande Dourados, and in the Laboratory for the evaluation of oilseed by-products, at the Center of Research Laboratories in Agroenergy and Environmental Conservation (LAPAC/FINEP), municipality of Dourados, state of Mato Grosso do Sul. All experimental procedures were performed according to ethical standards, approved by the institutional animal experimentation committee, according to protocol 004/2013-CEUA/UFGD.

Three crossbred steers, castrated, average body weight of 350 ± 23.5 kg, provided with a permanent ruminal cannula, were randomly distributed in two, non-contemporary 3x3 Latin square. Animals were housed in individual covered cubicles of 8 m² (2x4m), with dirt floor, containing individual feeders and drinkers.

The experimental period consisted of 90 days, divided into six periods of 15 days each, with the first 8 days of adaptation to diets and seven days for experimental collections. Diet was offered individually and mixed manually in the trough, and divided into two daily meals (8 am and 4 pm). The amount offered was adjusted daily, allowing a minimum of 10% surplus, throughout the experimental period.

The treatments consisted of diets based on corn silage; ground corn; inclusion of whole grains of canola, crambe or

soybean; urea and mineral mixture (Table 1).

Feces were collected on the 9th, 10th and 11th days at 6-h intervals, for each period, in the amount of 300 g/day. Collection of feces for the separation of canola, crambe and soybean grains occurred on the 12th, 13th and 14th days of the experimental period, at 6-h intervals. Samples were washed through 4-, 2- and 1-mm sieves, as described by Rennó et al. (2015). Canola, crambe and soybean grains were collected manually with the aid of tweezers, weighed, dried in a forced ventilation oven (55° for 72 h) and manually ground in a porcelain mortar and pestle.

Oilseed grain intake on a dry matter basis (kg/day) was calculated using the formula: $DMI_{gr} = (DMI \times \% \text{ grain in the diet}) / 100$. The concentration of oilseed grains excreted in feces (DM basis) was obtained by the equation: $DME = (\text{grain DM intake} - \text{grain DM excretion}) / \text{total excretion of feces} \times 100$. The excretion of DM from the grains (kg/day) was obtained by the equation: $DME = (\text{total excretion of feces} \times \% \text{ soybean grain in feces}) / 100$.

The chemical composition of the diets (Table 1), the feces and the grains recovered from feces was analyzed to determine the contents of dry matter (DM, method 967.03), mineral matter or ash (method 942.05, burning at 600 °C for 2 h), organic matter (OM = 100-ash), crude protein (CP, Nx6.25, method 981.10), ether extract (EE, method 920.29); as described by AOAC, (1990). Fractions of neutral detergent fiber (NDF), acid detergent fiber (ADF) were determined according to Van Soest et al., (1991), using 100 g/m² non-woven bags (TNT) using a fiber determiner (TE-149 - Tecnal®).

Table 1. Percent composition (%) of concentrates and chemical composition of experimental diets provided to cattle.

Ingredients	Oilseed grains		
	Canola	Crambe	Soybean
Corn silage	60.00	60.00	60.00
Ground corn	24.03	20.9	19.72
Canola grain	10.40	-	-
Crambe grain	-	14.10	-
Soybean grain	-	-	15.95
Urea	1.62	1.37	0.41
Mineral Mix	3.95	3.63	3.92
Chemical composition (g/kg)			
Dry matter	528.60	530.10	520.51
Organic matter	491.00	496.00	497.20
Crude protein	130.90	131.20	129.80
Neutral detergent fiber	321.10	385.30	325.60
Acid detergent fiber	167.10	224.80	172.26
Ether extract	55.10	86.00	58.90
Ash	85.90	82.50	88.10

On the 15th experimental day, samples of ruminal content were collected manually immediately before feeding and 2, 4, 6, and 8 hours after the diet was supplied, at the liquid/solid interface of the ruminal environment, which were filtered through a triple layer of gauze. A sample ruminal fluid was collected to determine pH and ammonia nitrogen (NH₃-N). Values of pH were measured immediately after the collection by means of a portable digital pH meter and for the determination of ammonia nitrogen, a 40 mL aliquot was separated, which was fixed with 1 mL H₂SO₄ 1: 1, stored in a glass with polyethylene lid, identified for further analysis. Determination of the levels of NH₃-N was carried out according to the INCT-CA N-007/1 method, described by Detmann et al., (2012). The coefficients of total apparent digestibility of dry matter (DM), organic

matter (OM), crude protein (PB), neutral detergent fiber (NDF) and ether extract (EE), were obtained from the relationship between the total nutrients retained and the fecal excretion.

Data obtained were teste for normality of residuals and homogeneity of variances by PROC UNIVARIATE of SAS, (2014) and evaluated by PROC MIXED according to the following model:

$$Y_{ijkl} = \mu + A_i + P_j + C_k + D_l + e_{ijkl}$$

Where: Y_{ijyk} = dependent variable, μ = overall mean, A_i = effect of animal (j = 1 to 3), P_j = effect of period (j = 1 to 6), C_k = effect of square (k = 1 to 2), D_l = effect of diet (l = 1 to 3) and e_{ijkl} = experimental error. The random effect of the model was characterized by: animal (A_i) and Latin square (C_k). The degrees of freedom were corrected by DDFM = kr. Data obtained were subjected to analysis of variance. Data were tested by Tukey's test using the PROC MIXED of SAS at

a significance of 5%.

RESULTS

Intake of the diets containing crambe grain reduced ($p=0.005$) the dry matter

intake by 21.02% (Table 2), consequently, steers showed lower intake of organic matter, crude protein and NDF. However, the inclusion of crambe grains provided greater digestibility for EE and NDF.

Table 2 – Intake (kg/day) and total digestibility (g/kg) of nutrients of steers fed canola, crambe and soybean grains.

Item	Oilseed grains			SEM ¹	P-value
	Canola	Crambe	Soybean		
<i>Intake (kg/day)</i>					
Dry matter	7.85 ^a	6.20 ^b	7.86 ^a	0.25	0.005
Organic matter	7.30 ^a	5.74 ^b	7.30 ^a	0.24	0.004
Crude protein	1.07 ^a	0.84 ^b	1.06 ^a	0.09	0.021
Neutral detergent fiber	2.89 ^a	2.34 ^b	2.55 ^{ab}	0.18	0.043
Ether extract	0.41	0.46	0.47	0.04	0.235
<i>Digestibility (g/kg)</i>					
Dry matter	0.645	0.645	0.645	0.01	0.997
Organic matter	0.673	0.669	0.683	0.01	0.613
Crude protein	0.665	0.705	0.696	0.03	0.829
Neutral detergent fiber	0.278 ^b	0.319 ^a	0.203 ^c	0.01	0.001
Ether extract	0.772 ^c	0.911 ^a	0.872 ^b	0.02	0.001

¹SEM: standard error of the mean

There was less ($p > 0.001$) NDF digestibility for diets containing soybean grains, although the intake of dry matter did not differ with the addition of canola ($p > 0.005$).

The chemical composition (Table 3) of soybean grain recovered from feces showed the lowest values for EE

(4.32%), crude protein (12.91%) and ADF (14.79%). The contents of crude protein in canola and crambe grains did not differ ($P > 0.005$) from each other; however, the EE values for crambe grains (26.46%) were higher ($P = 0.003$) than those determined for canola grains (16.78%).

Table 3- Intake, Excretion and Proximate Composition of grains excreted in feces of cattle fed canola, crambe and soybean grains.

Item	Oilseed grains			SEM ¹	P-value
	Canola	Crambe	Soybean		
Intake (kg/day)	0.82 ^b	0.88 ^b	1.26 ^a	0.05	0.015
Excretion (kg/day)	0.12 ^a	0.05 ^b	0.05 ^b	0.01	0.002
DME (%)	22.64	34.32	39.16	0.02	0.541
Chemical composition (%)					
Dry matter	94.95 ^a	94.86 ^a	91.52 ^b	0.63	0.043
Organic matter	96.91 ^b	97.43 ^b	99.73 ^a	0.31	0.001
Crude protein	29.13 ^a	36.51 ^a	12.91 ^b	3.12	0.005
Neutral detergent fiber	83.47 ^a	75.77 ^b	77.74 ^b	1.42	0.050
Acid detergent fiber	42.09 ^a	39.32 ^a	14.79 ^b	4.23	0.013
Ether extract	16.78 ^b	26.46 ^a	4.32 ^c	2.81	0.003
Ash	3.08 ^a	2.56 ^a	0.26 ^b	0.31	0.001

¹ SEM: Standard error of the mean

The inclusion of canola, crambe or soybean grains in diets changed the ruminal pH and the concentration of NH₃-N (Table 4). The content of NH₃-N in the ruminal fluid showed a reduction

with the inclusion of crambe grains, in contrast, the inclusion of canola grains resulted in high levels of NH₃-N in relation to other diets.

Table 4 – Mean values of ruminal pH and ammonia concentration (NH₃-N) of ruminal fluid from cattle fed canola, crambe and soybean grains.

Item	Oilseed grains			SEM ¹	P-value		
	Canola	Crambe	Soybean		Grain	Time	Interaction
pH	6.16 ^b	6.72 ^a	6.21 ^b	0.05	0.040	0.001	0.715
N-NH ₃ (mg/dL)	16.05 ^a	10.97 ^c	12.99 ^b	0.83	0.001	0.001	0.109

¹SEM: standard error of the mean;

DISCUSSION

Crambe grains contain 90 mol/g glucosinolates, [(S)-2-hydroxy-3-butenyl glucosinolate], which causes a reduction in intake, combined with the bitter taste, providing less acceptability by animals (Canova et al., 2015; Mendonça et al., 2015). Glucosinolate reduces intake after the third day of ingestion and activity of ruminal flora in cattle after the sixth day (Goes et al.,

2016). The higher proportion of unsaturated fatty acids (AGI) in the digesta increases the secretion of cholecystokinin (CCK), which can inhibit motility in the rumen and reticulum, thus reducing food intake (Nicholson & Omer, 1983).

Leduc et al. (2017) highlighted that the reduction in dry matter intake decreases the rate of passage through the rumen and increases nutrient digestibility, which corroborates data obtained for the

supply of crambe grains, which caused a reduction in DM intake of the animals and showed an increase for the NDF and EE digestibility coefficients.

The digestibility for EE ($p > 0.005$) for the inclusion of grains in the diet is in line with the chemical composition recovered from feces. Canola grain has $\pm 38\%$ EE and crambe grain $\pm 43\%$ (Goes et al., 2010, 2011), the EE content recovered from feces was 44.15% and 61.53%, respectively. However, among the studied diets, the treatment that included crambe showed greater ($p > 0.05$) digestibility compared to the others for this component.

Fresh soybean grain has a lipid matrix surrounded by an integumented membrane protecting the grain and regulating the moisture inside the endosperm (Mateus et al., 2018). Bassi et al. (2012) supplied soybeans, cottonseed and flaxseed for Zebu steers, and found no reduction in NDF digestibility of the diets, and this result was not verified in the present study, as the inclusion of soybeans decreased ($p < 0.05$) NDF digestibility. Rennó et al. (2015) tested the effects of including increasing levels of soybean grain in diets for beef cattle and high- and medium-production dairy cows and reported no differences between the levels of soybean grains in diets for NDF digestibility.

Even with a protected lipid matrix, lipids contained in soybean grain were used, since the EE content of the excreted grain was 4.32%. Therefore, it is possible to observe the use of lipids without negative effects on the ruminal environment of the animals, although they were not significant. The same was not observed for canola and crambe grains, which presented mean values of 16.78% and 26.46% EE, that is, there was greater use, however, as these grains

have a greater amount of total lipids, the recovery was greater.

According to Mizubuti et al. (2016), it has been observed a reduction in the digestibility of fiber compounds with the addition of lipid sources to the diets and the magnitude of reduction is related not only to the amount, but mainly to the type of fatty acid present in the supplement. In this sense, lipids rich in unsaturated fatty acids tend to cause greater depression in intake and digestibility, due to the toxic effect on rumen microflora, changing the permeability of the cell membrane, reducing the cell ability to regulate intracellular pH and nutrient uptake (Fiorentini et al., 2014).

The supply of lipid sources as whole grains results in slow release, altering the capacity of biohydrogenation, and influencing fiber digestibility, due to the negative effect that unsaturated fats available in the rumen can cause to fibrolytic bacteria (Rennó, et al., 2015; Coppock & Wilks, 1999). The inclusion of crambe grains into the diet provided the greatest digestibility for NDF and EE.

Goes et al. (2010) highlighted that crambe grain has a high soluble fraction, while Carlson et al. (1996) highlighted low degradability (44.5%) due to the presence of the hull, and the digestibility can be increased by dehulling. According to Wang et al. (1999) and Goes et al (2011), canola seed has a rigid capsule that impairs degradability; Santos et al. (2004) emphasized that the presence of condensed tannin causes the protection of DM of canola grains and consequent decrease of ruminal degradability, justifying the decrease of EE digestibility of animals fed canola grains.

Although oilseed grains are used for digestibility, the excess of non-metabolized nutrients provides an increase in energy expenditure for animal metabolism; or even increased financial costs for animal production. The greater feeding efficiency provided by the addition of grains provides a saving in anabolism, where preformed fatty acids do not require *de novo* synthesis from acetate, which would prevent the caloric increase associated with this metabolic pathway, which would require a greater amount of NADPH from the pentose cycle (Sousa et al., 2009).

Lipid composition of grains recovered from feces (Table 3) indicates that even with the protection offered by the hull, there was use of lipids contained in the grain. Rennó et al. (2015) pointed out that the lower values for EE of the recovered grains compared to the ingested levels indicate the use of lipids without negative effects on the rumen environment of the animals, as can be seen for soybeans that presented values of 4.32%; since the value of ether extract of fresh soybean grain is approximately 20%. Canola and crambe grains also showed a reduction in EE levels, when compared to the ingested levels.

The lowest content of CP presented by grains recovered from feces results in the solubilization of the external mucilage of the seed (Cortês et al. 2015); and that the CP content present in this fraction is readily soluble in water. Differences in concentrations of EE and CP between the diet and in grains recovered from feces can also indicate that the integrity of the seed coat has been damaged (Doucette et al., 2001); which can justify the chemical composition values listed in Table 3.

Animals fed a diet containing crambe grain showed ($p < 0.05$) a potential of

hydrogen with values close to neutrality (7.0) in relation to diets containing soybean and canola grains. Nonetheless, the test-maintained pH values above 5.58, which is not only related to the good microbial growth and balance of its population, but the metabolic, systemic state and ability to catabolize and/or excrete certain metabolites as changes in the main volatile fatty acids.

According to Vargas et al. (2002), inclusion of oilseed grains in the diet positively influences rumen pH, negatively affecting dry matter intake and rumen fermentation. Thus, the high content of ether extract for the diets provided a buffering effect, which justifies the stability observed for canola and soybean and the slight increase found with the inclusion of crambe grain. Although the values were close to what was established by Hoover (1986), with a limit of 6.2. According to Van Soest (1994), declines in rumen pH are responsible for the partial inhibition of fiber degradation, as they impair microbial growth and provides less accumulation of volatile fatty acids, the main factor responsible for raising the pH.

The ruminal pH influences the changes that lipids undergo in the rumen, changing lipolysis and biohydrogenation at lower pH (Van Nevel & Demeyer, 1996), resulting in escape of unsaturated fatty acids to the abomasum.

The content of rumen ammonia $\text{NH}_3\text{-N}$ (Table 4) is an important factor, since ruminal microorganisms use it as a source of nitrogen for protein synthesis (Ribeiro et al., 2001). Lipid supplementation allows a reduction in the concentration of $\text{NH}_3\text{-N}$ in the rumen fluid due to rumen defaunation (Ikwuegbu & Sutton, 1982; Vargas et al.,

2002; Nguyen et al., 2003; Eifert et al., 2005).

The concentration of rumen NH₃-N is fundamental for microbial growth and efficiency (Van Soest, 1994), so that there is no limitation in microbial fermentation, the minimum concentration of NH₃-N should be around 5 mg/100 mL ruminal fluid (Costa et al., 2015), and 23 mg NH₃-N/mL for maximum microbial synthesis (Sampaio et al., 2009). Thus, it is highlighted that the values observed in all treatments are in accordance with the values indicated to not limit fermentation and for maximum microbial synthesis, Whole grains of oilseeds can be included in the diet for beef cattle, however the addition of crambe grain reduces the intake of dry matter, and provides greater intake of ether extract, which improves its digestibility. Chemical composition of grains recovered from feces indicates a better use of the ether extract and crude protein for the soybean grain, and the canola and crambe grains presented a similar composition when recovered from feces.

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