Original Article

THE CRAMBE (*Crambe abyssinica* Hochst) BYPRODUCTS, CAN BE USED AS A SOURCE OF NON-DEGRADABLE PROTEIN IN THE RUMEN?

COPRODUTOS DE CRAMBE (*Crambe abyssinica* Hochst) PODEM SER UTILIZADOS COMO FONTE DE PROTEINA NÃO DEGRÁDAVEL NO RÚMEN?

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ABSTRACT: To evaluated the chemical composition and ruminal degradability of crambe byproducts (meal and crushed) and proteic supplements formulated with crushed crambe (0; 2.5; 5; 10 and 15%); five crossbred steers with average weight of 485±14 kg, were used. All the animal were kept in individual paddocks of 0.25 ha on *Urochloa brizantha* (syn. *Brachiaria brizantha*). It was observed a greater soluble fraction, higher effective degradability at 5%/h and higher degradation rate "c" and, consequent, lower indigestible fraction for crambe crushed ground in the sieve of 3 mm. The effective degradability at 5%/h was lower for the crambe crushed (55.42) in relation to the meal (48.80). The diet with 5% of inclusion of crambe showed higher effective degradability for dry matter (54.86%) and lower fraction "I" (30.64%) associated with higher fractions "c" and "b". Crambe ground in sieves of 1 and 3 mm mesh presented the highest degradability. Crushed crambe showed higher ruminal degradation than crambe meal; the crambe byproducts possibility can be use as a source of non-degradable protein in the rumen.

KEYWORDS: Crambe meal. Crushed crambe. *In situ* degradability.

INTRODUCTION

Oilseeds are used in ruminant diets due to their high concentrations of lipids, and composition of fatty acids, rich in unsaturated fatty acids (ω-3 and ω-6), and because they present a slow release of oil, due to chewing, which results in small fractions coming into the rumen (CIEŚLAK et al., 2013).

Crambe (*Crambe abyssinica* Hochst) has been widely used for the extraction of oil for biodiesel production. Vegetable oils used for biodiesel production can be extracted from oilseeds through two different processes: with the use of solvents or by pressing. The waste from the use of solvents for oil extraction is meal and from pressing, crushed grain. The seed has between 46 and 58% CP with 44% EE (Souza et al. 2009; Goes et al., 2010). Crambe meal contains about 47.4 µmol/g of glucosinolates [(S)-2-hydroxy-3-butenyl glucosinolate]; and it´s toxic (11-24,3 mol/g) to many organisms and impairs the activity of rumen flora in cattle after six days of ingestion (TRIPATHI; MISHRA, 2007).

Considering the high nutritional potential of this oilseed, and if crambe products can be used in ruminant nutrition, the present study evaluated by the *in situ* technic the kinetic patterns of ruminal degradation of dry matter and crude protein of crambe byproducts, and, degradability of supplements with inclusion of crushed crambe replacing soybean meal.

MATERIAL AND METHODS

The experiments were conducted at the Sector of Ruminant Nutrition and Animal Nutrition Laboratory of the Federal University of Grande Dourados. In both experiments we used, crossbred steers with average weight of 485±14 kg, cannulated in the rumen were kept in individual paddocks (0.25 ha) of *Urochloa brizantha* (syn. *Brachiaria brizantha*) cv. Marandu (7047.85 kg of DM/ha); receiving daily, in the morning (8:00h), 8 g/kg body weight of supplement containing crushed crambe (Table 1).

Experiment 1

Crambe byproducts (crushed and meal) were evaluated in two trials; on the first trial we evaluated the ruminal degradability of crambe

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crushed at different particle size (1, 3 and 5 mm) and the second trial were evaluated the ruminal degradability of crambe meal and crambe crushed grounded at 3mm. The chemical composition of crambe byproducts presents in Table 2. In both trials the crushed crambe was obtained by cold pressing.

Table 1. Percentual to the experimental supplements.

Ingredients (g/kg)	C0	C2.5	C5	C10	C15
Crushed crambe	-	25	50	100	150
Soybean meal	150	125	100	50	-
Rice meal	400	400	400	400	400
Corn meal	376	375	373	369	366
Urea	3.5	5.2	7.0	10.5	14
Salt	10	10	10	10	10
Limestone	25	25	25	25	25
Sulfur	10	10	10	10	10
Dicalcium phosphate	15	15	15	15	15
Premix[2]	10	10	10	10	10

[1]C00=supplement without crambe crushed; C2.5= supplement with 2.5% crambe crushed; C5= supplement with 5% crambe crushed; C10= supplement with 10% crambe crushed; C15= supplement with 15% crambe crushed.[2]Calcium: 120.00 g. phosphorus: 88.00 g. iodine: 75.00 mg. manganese: 1300.00 mg. sodium: 126.00 g. selenium: 15.00 mg. sulfur: 12.00 mg. zinc: 3630.00 mg. cobalt: 55.50 mg. cooper: 1530.00 mg e iron: 1800.00 mg.

Table 2. Chemical composition (g/kg) and in vitro dry matter digestibility (IVDMD) (%) of crambe byproducts.

Feedstuffs	DM	OM	CP	EE	NDF	ADF	LIG	IVDMD
Crushed Crambe	943.0	952.2	261.9	182.7	302.3	194.4	84.0	62,04
Crambe Meal	899.0	931.9	350.0	41.0	350.0	242.0	113.0	58,61

DM = Dry matter, OM = organic matter, CP = crude protein, EE=ether extract, NDF = neutral detergent fiber, ADF = acid detergent fiber, MM = mineral matter, LIG = lignin.

The feedstuffs were analyzed to dry matter (DM – method 930.15); ash (Ash - method 942.05) and the organic matter (OM = 100 - ash); crude protein (CP – method 976.05, N X 6.25) and ether extract (EE – method 920.39), following the methodologies of AOAC (2006). ADF contents were obtained following method described by Van Soest and Robertson (1985). Lignin content was obtained by oxidation with potassium permanganate (VAN SOEST; WINE, 1968). For analysis of NDF, samples were treated with heat stable alpha amylase without sodium sulfite and corrected for ash residue (MERTENS, 2002).

The feedstuffs were dried at 65ºC for 24 hours, removed, and weighed. After weighing, the food was packed in TNT bags (TNT -100 g/m^2) in size 5.0 x 5.0 cm, respecting the relationship 20 mg / cm² (CASALI et al. 2009). The samples were prepared and incubated according to Nocek (1988) and Huntington & Givens (1995). TNT Bags were introduced directly into the rumen, in decreasing order of 48, 36, 24, 12, 8, 4, 2 and 0 hours, in triplicates per animal/incubation time, according to NRC (2001); and . removed all at once and rinsed in tap water, until clean. The remaining residues from the incubation were oven dried at 65ºC for 48h and stored for later analysis to determine the variables studied.

The disappearance of dry matter and crude protein (N x 6.25) were based on the weight difference between the incubated material and the residues after incubation. To estimate the kinetic parameters of DM and CP, we used the first-order asymptotic model described of Ørskov e McDonald (1979): $PD = a+b(1-e^{-ct})$. Where: PD=potential rumen degradability; 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 is calculated according to Ørskov & McDonald (1979): I = 100-(a+b). And the effective degradability (ED) is calculated with the following equation: ED = a + [(b*c)/(c+K)]; where K=passage rate of solids from the rumen, herein defined as 2, 5 and 8.0% per hour (h), which can be attributed to the low, medium and high dietary intake. After fitting the data to the model and using the disappearance value obtained at the time zero (a'), we estimated the colonization time (CT) as proposed by Patiño et al. (2001), where the

parameters a, b and c were estimated by the Gauss Newton algorithm: TC = [-ln(a'-a-b)/c].

Experiment 2

The inclusion levels of crambe crushed in the concentrates was 0; 2.5; 5.0; 10 and 15% (Table 1). Three samples were taken of each concentrate, which were placed in plastic bags, identified and stored at -20°C.

For in situ degradability, the protein supplements were ground in 3 mm sieve, and then dried at 65ºC for 24 hours and weighed. Bags were introduced directly into the rumen, in descending order of 48, 36, 24, 12, 8, 4, 2 and 0 hours, in triplicate per animal/incubation time, according to NRC (2001). Each supplement was incubated in an animal receiving the same treatment. The rest of the methodologies used in the in situ degradability trial of protein supplements were similar to those in the experiment 1.

Statistical analysis

The animals were distributed in a randomized latin square design (5x5); and **t**he degradation curves of dry matter and crude protein of feedstuffs evaluated, for each animal used, were subjected to fit by the respective models using the PROC NLIN of SAS 9.2. The in vitro dry matter digestibility was analyzed by polynomial regression PROG REG of SAS 9.2, with significance level of 5%.

RESULTS

Experiment 1 - trial 1

The kinetic parameters of in situ degradation for different particle sizes of crushed crambe were similar, with intermediate degradability for DM and CP (Table 3).

Table 3. Kinetic parameters of *in situ* degradation of crushed crambe in different particle sizes

	Parameters*			I (%)	Effective degradability(%.h^{-1})				CT (min)
Dry matter	a (%)	b (%)	c(%/h)		2	5	8	r^2	
1 mm	27.86	22.24	0.08	49.90	45.31	41.16	38.65	88.75	342
3 mm	28.16	27.01	0.17	44.82	47.83	43.21	40.80	97.04	346
5 mm	26.20	23.37	0.07	50.42	44.11	39.46	36.73	91.49	353
Crude protein									
1 mm	25.64	36.89	0.25	37.47	59.71	56.24	53.43	90.90	301
3 mm	31.55	28.56	0.31	39.89	58.36	56.11	54.21	88.28	271
5 mm	34.33	28.67	0.19	37.00	59.15	55.42	52.88	74.27	314

*a=soluble fraction; b= potentially degradable fraction; c= degradation rate of fraction b. I=indigestible fraction. CT = Time of colonization (minutes)

It was observed a greater soluble fraction, higher effective degradability at 5%.h^{-1} and degradation rate "c", consequent, lower indigestible fraction for feedstuffs grounded of 3 mm (28.16%, 43.21% and 44.82%, respectively).

The crushed crambe grounded of 3 mm showed the highest degradation rates, and greater potentially degradable fraction of DM. For CP, the ground in the 5 mm demonstrated the highest soluble fraction, which did not result in higher effective degradability. The soluble fraction observed for the ground of 5 mm was 34.33%, but this value not correspond a greater effective degradability at 5%.h^{-1} (55.42%), lower than that found for the sieves of 1 and 3 mm.

Experiment 1 - trial 2

The kinetic parameters of *in situ* degradability, there was a higher percentage for the fraction "a" and fraction "I" for the DM of the crushed crambe (Table 4). In turn, the meal showed higher percentage of the fractions "b" and "c" (%.h^{-1}). Also, we observed a low degradability at 5%.h^{-1} for both byproducts.

The crushed presents a higher value for fraction "a" and "c", by CP, and the meal have a higher percentage of the fractions "b". The effective degradability at 5%.h^{-1} was lower for the crushed crambe (55.42%) in relation to the meal (48.80%).

Experiment 2

The crushes crambe reduces the NDF of supplements, but increases the EE (Table 5).

The diet with 5% of inclusion showed higher effective degradability for dry matter (54.86%) and lower fraction "I" (30.64%) associated with higher fractions "c" and "b". The lower soluble fraction "a" and lower effective degradability at 5%.h^{-1}, were verified in the 10% of

inclusion, with 6.68 and 49.08% respectively for DM (Table 6).

Table 4. Kinetic parameters of *in situ* degradation crambe byproducts.

	Parameters*				Effective degrabability (%.h^{-1})				
Dry matter	a (%)	b (%)	c(%/h)	I (%)	2	5	8	r^2	CT (min)
Crushed	26.20	23.17	0.07	50.63	43.96	39.35	36.64	91.49	353
Meal	18.94	31.39	0.11	49.67	29.17	38.26	34.95	99.14	352
Crude protein									
Crushed	34.33	28.67	0.19	37.00	59.15	55.42	52.88	74.27	316
Meal	20.68	43.24	0.10	36.08	37.44	48.80	44.05	94.44	364

*a=soluble fraction; b= potentially degradable fraction; c= degradation rate of fraction b. I=indigestible fraction. CT= colonization time in minutes

Table 5. Chemical composition of supplements contents crushed crambe

Nutrients[1]	Levels of crushed crambe (%)					Average	MSE[2]	P<F[3]
	0.0	2.5	5.0	10.0	15.0			
DM	926.9	918.5	936.7	923.6	922.0	925.5	0.30	ns
OM	863.0	867.2	873.9	876.7	874.4	871.0	0.33	ns
CP	153.4	148.6	155.0	155.7	150.0	152.5	0.35	0.244
EE	96.0	96.3	99.1	99.8	114.3	101.1	0.38	0.145
NDF	518.7	369.4	421.0	363.9	390.8	412.5	1.28	0.003
LIG	47.2	25.7	49.1	28.1	31.3	35.9	0.21	0.072
TCHO	636.5	647.1	618.0	631.2	620.0	630.9	0.69	ns

[1]DM: Dry Matter (g/kg); OM = organig matter (g/kg); CP: crude protein (g/kg); EE: ether extract (g/kg); NDF: neutral detergent fiber (g/kg); LIG: lignin (g/kg); TCHO: total carbohidrates (g/kg); [2]MSE: mean standard error. [3]ns: not significance; NDF: Y = 486.3 – 25.0 x (r^2 = 0.33).

Table 6. Kinetic parameters of *in situ* degradation of experimental supplements

Crambe Inclusion	Parameters*				Effective Degradability (%.h^{-1})				
Dry matter	a (%)	b (%)	c(%/h)	I (%)	2	5	8	r^2	CT (min)
00	13.49	54.19	0.1161	32.32	59.71	51.36	45.57	97.40	369
2.5	8.49	58.61	0.1283	32.90	59.19	50.66	44.59	97.67	367
5.0	10.54	58.82	0.1528	30.64	62.55	54.86	49.15	97.90	357
10.0	6.68	56.60	0.1492	36.72	56.59	49.08	43.53	98.00	356
15.0	11.44	52.26	0.1707	36.30	58.22	51.86	47.03	98.30	343
Crude protein									
00	20.32	48.43	0.0808	31.24	68.61	59.15	50.24	52.78	384
2.5	18.67	44.30	0.1661	37.03	62.97	58.20	52.71	73.79	335
5.0	31.20	44.49	0.0398	24.31	73.15	60.80	50.90	81.99	421
10.0	23.63	42.98	0.1398	33.40	66.60	61.22	55.28	90.39	344
15.0	29.52	39.83	0.2762	30.65	69.35	66.66	63.25	95.26	298

*a=soluble fraction; b= potentially degradable fraction; c= degradation rate of fraction b. I=indigestible fraction; CT = colonization time in minutes

For crude protein, we observed greater soluble fraction "a" for the diet C5 (31.20%) and lower fraction "I" (24.31%), which did not promote the greater effective degradability at 5%.h^{-1}, which was observed for 15% of inclusion (66.66%).

DISCUSSION

Experiment 1 – trial 1

The results of *in situ* degradability kinetics indicated little variation in parameters between the three sieves (1, 3 and 5 mm) (Table 3). Ruminant digestive processes are influenced by the particle size of the feed and its flow through the rumen. Despite having ground the feedstuffs evaluated to increase the contact area, all feedstuffs showed an intermediate degradation with similar colonization times.

Physical and chemical characteristics interfere with feed degradability, where lower contents of NDF are related to greater capacity for water retention (QUEIROZ et al. 2010). The water holding capacity strongly affects the colonization by microorganisms (DU et al., 2010) and can influence the filling effect, altering the passage rate.

Moreover, several alternatives have been evaluated to decrease protein degradability in the rumen and thereby enable better availability of amino acids for intestinal digestion (SANTOS et al., 2004). According to Carlson et al. (1996) and Anderson et al. (2000), crambe is a good source of cysteine, methionine, lysine, alanine, glutamate, aspartate and threonine, thus manipulating the amino acid profile in the duodenum may be interesting, but this can reduce the contribution of nitrogen for microbial synthesis. The kinetics of crude protein degradation corresponds to the kinetics of dry matter. The soluble fraction for the byproduct grounded at 5 mm was 34.33%, however this value does not correspond to a higher effective degradability at 5%.h^{-1}. This indicates that the particle size does not influence the crude protein degradability, and the soluble fraction of this nutrient was high for all particle sizes used.

Experiment 1- trial 2

Considering the chemical composition of the crushed crambe, Souza et al. (2009) observed 317.9 g/kg of CP and Goes et al. (2010) found CP value of 528.0 g/kg, higher than that observed in this study.

To the kinetic parameters of *in situ* degradation for dry matter, both byproducts showed a low effective degradability at 5%.h^{-1}. Goes et al. (2010) examined the crushed crambe reported 60.43% of effective degradability at 5%.h^{-1}. The low degradability may be associated with low values presented by the potentially degradable fraction (b), similar to that found by Brás et al. (2014), which resulted in high values for the undegradable fraction, and low degradation rate. Nevertheless, crushed crambe showed higher soluble fraction for dry matter, as also observed by Goes et al., (2010).

The difference in kinetics of degradation between byproducts can be related to the pressing process. According to Beran et al. (2005), pressing of seeds cause compaction, which after milling may result in smaller particles and facilitate the solubilization. Brás et al. (2014) pointed out that the degradability presented, may be associated with rumen not be propitious to microbial action, due to anti-nutritional factors, such as erucic acid.

The difference between the values for fraction "a" may be related to the hydration capacity of the source. Higher solubility values are associated with reduction of potentially degradable fraction. In addition to the water-holding capacity, physical parameter is critical to the determination of degradability, including the fiber content (QUEIROZ et al., 2010). Foods with less density retaining feature between the cell wall matrix which can retain the free water in the rumen (SEOANE et al., 1981).

Carlson et al. (1996) evidenced that hull and grain have low degradation (44.5 and 57.3%), and that the degradability of dehulled crambe meal is similar to soybean meal. Liu et al. (1994) analyzed the partially hulled crambe and found low ruminal degradation, which was associated with high content of EE. In the present study, even with higher values of EE, the crushed showed a behavior similar to that of the meal, indicating that oil content did not interfere CP degradability.

In relation to the colonization time, we observed close values for dry matter (353and 352minutes). However, for crude protein, the colonization time for crambe crushed was shorter than for the meal (316 and 364 minutes), which may explain the higher values of degradation rate and, consequently, higher values of degradability. The water holding capacity has a substantial impact on the colonization by microorganisms thus explaining the values obtained for the crambe crushed.

The short colonization time for crude protein along with higher soluble fraction and effective degradability of crushed crambe shows that this feedstuff can be used for animal feeding as a source of protein for rumen microorganisms, as it is readily degraded in the rumen at a short time.

Experiment 2

When studied the ruminal kinetics of crushed crambe, Brás et al. (2014) and Goes et al. (2010) reported a mean effective degradability of DM ranging from 54.00 to 60.43%, similar to the value verified in the present study, but the soluble fraction of concentrates with crambe crushed was lower. Differences in soluble fraction of DM may arise from the oil extraction process, in which some heating occurs during pressing, making the fraction less available, which can be related to the degradation rate "c". The intermediate degradability presented by the crambe crushed can be because the rumen environment and microbial activity, leading to reduced intake, weight loss, due to antinutritional factors, but the dry matter colonization time was shorter for the highest concentration of crambe. Primo (2013) analyzed microbial activity in the rumen fluid of steers supplemented with the same levels of this study found a reduction in 25% when 15% of crushed crambe was added in diet.

According to Liu et al. (1994), crushed crambe presents high ruminal degradability, small proportion of slowly degradable protein and degradation rate of 11.4%/h. The highest solubility presented by supplements may be associated with the presence of urea (Table 1).

Carlson et al. (1996) and Anderson et al. (2000), showed that amino acid profile of crambe maybe participate in the hepatic gluconeogenesis in ruminants, indicating that the manipulation of the amino acid profile in the duodenum can be interesting because protein sources of low degradability enable such manipulation, but this can reduce the contribution of nitrogen for microbial synthesis.

Higher degradation may lead to oil availability in the rumen, however, the supplementation of lipids in the diet for ruminants can bring problems associated with the reduced degradation of the fibrous fraction of the diet and changes in rumen metabolism. The crambe is rich in polyunsaturated fatty acids, which may be biohydrogenated by rumen protozoa and bacteria providing increased energy availability (GOES et al. 2010).

CONCLUSIONS

Crambe byproducts possibility can be use as a source of non-degradable protein in the rumen.

Crambe crushed showed higher ruminal degradation than crambe meal; and the feeds grounded at 3 mm mesh presented the highest degradability values for CP.

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RESUMO: Para se avaliar a composição química, degradabilidade ruminal, o tempo da colonização da torta e do farelo de crambe e de suplementos protéico compostos de torta de crambe (0; 2,5; 5,0; 10 e 15%); foram utilizados cinco novilhos mestiços, com peso médio de 485 ± 14 kg. Todos os animais foram mantidos em piquetes individuais de 0,25 ha em pastagens de *Urochloa brizantha* (syn. *Brachiaria brizantha*). Observou-se uma fração solúvel maior, maior degradabilidade efetiva para a taxa de passagem de 5% / h e maior taxa de degradação "c" e, consequentemente, menor fração indigerível para a torta de crambe moído na peneira de 3 mm. A degradabilidade efetiva a 5% / h foi menor para a torta de crambe (55,42) em relação ao farelo (48,80). A dieta com adição de 5% de torta de crambe apresentou maior degradabilidade efetiva da matéria seca (54,86%) e menor fração "I" (30,64%), associada com as frações mais elevadas "c" e "b". A torta de Crambe moída em peneiras de 1 e 3 mm de diâmetro apresentaram os maiores valores de degradabilidade. A torta de apresenta maior degradação ruminal que o farelo de crambe. Os coprodutos de crambe possivelmente podem ser usados como fonte de proteína não degradada no rúmen.

PALAVRAS-CHAVE: Cinética de degradação. Degradabilidade *in situ*. Farelo de crambe. Torta de crambe.

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