Evaluation of nutrient intake in sheep fed with increasing levels of Crambe meal (*Crambe abyssinica* Hoscht)

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Abstract. *The objective of this study was to evaluate the effects of increasing levels of crude protein (CP) substitution of the concentrate by CP of crambe meal (CM) (0, 25, 50 and 75% dry matter basis) on consumption of nutrients. Four rumen fistulated and castrated sheep (18 months old on average and initial body weight of 50 kg) were used distributed in a 4 x 4 Latin square design with 4 treatments and 4 experimental periods (repetitions). Diets were balanced to meet requirements for minimum gains of 50 grams/animal/day with approximately 14% CP and 70% total digestible nutrients (TDN). The roughage to concentrate ratio was 50:50. Animals had free access to food, water, and mineral mixture. Each experimental period contained 14 days, seven days for the adaptation of the animals to the diet and the experimental conditions and seven days for sample collection. Foods supplied and leftovers were quantified daily to measure the individual intake of dry matter (DM), crude protein (CP), ethereal extract (EE), neutral detergent fiber corrected for ashes and protein (NDFap), non-fibrous carbohydrate (NFC), and total carbohydrate. The intake was expressed in kilograms per day (kg/day), grams per kilogram of body weight (g/kg BW), and grams per kg of metabolic weight (g/kg* $BW^{0.75}$*). The results were submitted to analysis of variance and regression study at 5% of significance. There was a linear increasing effect (* $P < 0.05$*) on EE consumption (kg/day), which can be mainly attributed to differences in diets formulation. In addition, a linear decreasing effect (* $P < 0.05$*) was observed for NFC intake, as well as DM, OM, EE, NDFap, NFCap, and TDN (* $P < 0.05$*). However, no effect was observed for digestibility of CP (* $P > 0.05$*). Thus, it can be concluded that crambe meal can be used to feed sheep without compromising feed intake of the animals.*

Keywords. *Crambe bran, intake, nutrients, sheep.*

Introduction

The intensification of animal production increases productivity. However, most diets fed to animals are based on corn and soybean as energy and protein sources, respectively, increasing the competition with human feed source as well as among monogastric and ruminant animals. Therefore, the use of alternative feedstuff is an important strategy to promote sustainability of the production chain.

The current systems to formulate ruminant diets rely on information regarding the proportions of each feedstuff. In addition, information on digestion rate is required to synchronize energy and nitrogen availability in the rumen to maximize microbial efficiency as well as reducing energy loss during ruminal fermentation. Altogether, improving economic aspects of animal feeding (Goes et al. 2010).

Among alternative oilseed with potential for biodiesel production, we highlight the Crambe (*Crambe abyssinica* Hochst) (Goes et al. 2010). Interest in the production of Crambe was aroused due to its capacity to produce oil (26% to 38% of the grain) (Echevenguá 2007). Crambe meal, on the other hand, is obtained from the process of oil extraction using the chemical solvent hexane (CH_3 (CH_2) $_4CH_3$) (Mizubuti et al. 2009). According to Souza (2014), most of this co-product still does not have a market destination and ended up being used to generate energy in the boilers or discarded in the environment serving as organic fertilizer on crops.

Crambe meal and bran have more than 20% of crude protein as well as high levels of cellulose and hemicellulose, which suggests that they could be classified as a protein concentrate for ruminants. If used as animal feedstuff, these co-products could potentially generate greater interest value, increase the portfolio of feedstuff for animal raising, and reduce waste accumulation around biodiesel plants.

Crambe is known to have an antinutritional factor which is the glycosinolates, produced in the secondary metabolism of brassicaceous plants. However, the development of the ruminal microbiota in ruminant animals confers to them great tolerance to glycosinolate intake, making adult animals more resistant to this antinutritional factor as compared to young animals (Tripathi and Mishra 2007).

Because Crambe meal is a relatively new co-product available on the market, it is still underutilized and further studies are needed, especially with small ruminants, since most studies are carried out with the cattle. Therefore, the objective of this study was to evaluate the effect of increasing levels (0%, 25%, 50% and 75%) of substitution of crude protein (CP) from the concentrate by the CP of Crambe meal (CM) on consumption of nutrients in sheep.

Materials and methods

Place of experimentation and ethical consideration

The experiment was conducted between September and November of 2015 at the Ruminants Laboratory of the Moura Experimental Farm (FEM) from the Federal University of Jequitinhonha and Mucuri Valley (UFVJM), located in Curvelo, Minas Gerais State, Brazil (18°45'21" South, 44°25'51" West, and 632 m altitude). Analyzes were performed in the Animal Nutrition

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Laboratory from the UFVJM – JK Campus, located in Diamantina, Minas Gerais, Brazil. The experimental procedures were approved by the Committee on Ethics in the Use of Animals (CEUA) of the UFVJM, registered under the protocol number 002/2014 (September 17th, 2014).

Animals and diets

Four male lambs (18 months old and initial body weight of 50 kg), with unknown breed and castrated, were selected from the herd and fistulated in the rumen according to the procedure described by Gomes (2009). They were used in this study six weeks after surgery. During the pre-experimental period, animals were orally treated against endoparasites with anthelmintic 10% albendazole base.

The lambs were randomly distributed in four metabolic craters of 1.2 m x 0.6 m, equipped with two troughs (one for diet and other for mineral mixture), as well as a drinking fountain for fresh and clean water. The crates were cleaned daily.

The experimental design was a 4 x 4 Latin square (4 treatments and 4 periods). Each period was composed of 14 days, the first seven days were the adaptation period to the diet and experimental conditions, and the following seven days were for sample collection. The adaptation period was considered sufficient since the animals were fed the same roughage used in the experiment and housed in the metabolic crates at least seven days before the start of the adaptation period.

The treatments consisted of diets containing increasing levels of inclusion of CP from CM as a replacement for concentrate CP (Table 1). The following treatments were randomly distributed among animals: 0 = 0% CP substitution of the concentrate for CP from CM (control); 25 = 25% CP substitution of concentrate for CP from CM; 50 = 50% CP substitution of concentrate for CP from CM; 75 = 75% CP substitution of concentrate for CP from CM.

Table 1 – Total diet formulations

Feedstuff (% of dry matter)	Diet			
	0	25	50	75
Ground corn	28.0	28.0	25.7	16.7
Soybean meal	16.8	12.8	4.1	3.1
Wheat bran	5.2	0	0	0
Crambe meal	0	9.2	18.3	27.4
Soybean oil	0	0	1.6	3.1
Urea-AS (9:1)[1]	0	0	0.5	0
Mineral mix[2]	2.0	2.0	1.8	1.7
Corn silage	48.0	48.0	48.0	48.0

[1]/ Urea-AS (9:1) – urea mixed with ammonium sulfate at 9:1 ratio. [2]/ Mineral mix composition (kg of product): 3800 mg of zinc, 147 g of sodium, 1300 mg of manganese, 40 mg of cobalt, 1800 mg of iron, 590 mg of copper, 18 g of sulfur, 15 g of selenium, 80 mg of iodine, 20 mg of chrome, 300 mg of molybdenum, 120 g of calcium, 870 mg of fluorine, and 87 g of phosphor.

The Crambe meal donated by Caramuru Alimentos S.A and used without previous treatment. Diets were balanced according to NRC (1985) for minimum gains of 50 grams/animal/day with approximately 14% of CP and 70% of total digestible nutrients (TDN; Table 2). Diets were supplied *ad libitum*, twice a day, always at 06:30 and 14:30. The roughage to concentrate ratio of the diets was approximately 50:50, dry matter (DM) basis (Table 1), with corn silage as the exclusive source of roughage. Forage and concentrate were manually mixed right before feeding the animals. All animals were weighed on the first day of each experimental period to determine the amount of feeding as a body weight percentage (% BW). Feeding was adjusted during the experimental period to allow 20% of leftovers.

Table 2 – Nutritional composition of diets

Nutrient (% dry matter)[1]	Diet				Crambe meal
	0	25	50	75	

Dry matter	89.73	89.77	90.46	90.95	91.32
Organic matter	93.35	92.34	92.17	88.85	91.46
Crude protein	13.82	14.23	14.88	15.18	31.75
RDP	64.83	65.21	69.82	68.44	70.40
NRDP	38.49	37.87	33.13	34.45	29.60
NDIN	15.12	16.19	17.28	17.80	20.60
ADIN	2.88	2.97	3.15	3.30	2.55
Ethereal extract	2.91	2.77	4.06	5.15	0.89
NDFap	32.15	32.73	34.26	35.86	29.32
NDFpd	21.21	20.23	19.86	19.58	6.28
ADF	13.53	14.57	15.67	17.00	17.44
Lignin	2.71	3.12	3.63	4.20	6.88
NDFi	10.94	12.49	14.40	16.28	23.03
ADFi	4.42	5.55	6.88	8.21	15.32
NFCap	43.79	42.61	40.06	35.66	29.50
Total carbohydrates	75.94	75.34	74.32	71.52	58.82
TDN	69.70	69.02	68.41	65.02	58.61[2]

[1]/ RDP: ruminal degradable protein as percentage of crude protein (CP); NRDP: non ruminal degradable protein as percentage of CP (valor estimated based on Brazilian Table of Feed Composition for Cattle - CQBAL 3.0); NDIN: Neutral detergent insoluble nitrogen (% total Nitrogen); ADIN: acid detergent insoluble nitrogen (% total Nitrogen); NDFap: Neutral detergent fiber corrected for ashes and protein; NDFpd: Neutral detergent fiber potentially digestible; NDFi: Indigestible neutral detergent fiber; ADFi: Indigestible acid detergent fiber; NFCap: Non fibrous carbohydrate corrected for ashes and protein; TDN: Total digestible nutrients.
[2]/ Estimated based on literature values.

Measurements and sample collection of food, leftovers and feces

Corn silage and concentrate supplied as well as leftovers were weighed daily to estimate individual consumption as a percentage of body weight (% BW), kilograms per day (kg/day), grams per kilogram of body weight (g/kg BW), and grams per kilogram of metabolic weight (g/kg $BW^{0.75}$).

Diet components (corn silage and concentrate) were sampled on the 1st day of each experimental period, packed in plastic bags, identified by treatment and period, and stored at -20ºC. Leftover samples were collected daily in order to obtain two composite samples per animal per period. After thawing, these composite samples and diet components were homogenized per treatment and per period, dried in a forced ventilation oven at 65ºC and ground in 1.0 mm sieve mills for further laboratory analysis.

Total digestible nutrient consumption and total apparent digestibility coefficients of the nutrients, were determined by means of a digestibility test carried out from the 8th to the 12th day of each experimental period. Feces were daily collected using collection bags adapted to the animals. They were weighed. A sample of 20% of total feces excretion was collected and processed the same way as leftovers and diet components. Samples for each animal in each period were homogenized to form composite samples for subsequent chemical-bromatological analyzes.

Chemical analyzes and calculations

Samples of food, leftovers, and feces were analyzed to quantify DM, mineral matter (MM), organic matter (OM), CP, and ethereal extract (EE) according to AOAC (1997). Neutral detergent fiber (NDF) was determined according to Mertens (2002) using thermostable amylase (Termamyl 120L, Novozymes) and corrected for ash and protein (NDFap). The Ankom® system was used for NDF evaluations, with a modified bag (5.0 x 5.0 cm, 100 µm porosity), which was made using non-woven fabric (TNT - 100 g/m^2). Acid detergent fiber corrected for ashes and protein (ADFcp), acid detergent insoluble nitrogen (ADIN), neutral detergent insoluble nitrogen

(NDIN), and lignin (72% sulfuric acid) were estimated according to Robertson and Van Soest (1981) sequential method following the protocol presented by Licitra et al. (1996).

Non-fibrous carbohydrates corrected for ashes and protein (NFCap) were estimated according to Hall and Akinyode (2000), using the formula:

$$NDFap = 100 - [(\% CP - \% Urea CP + \% Urea) + (\% NDFap) + \% EE + \% MM].$$

Total digestible nutrients were estimated following the equation proposed by the NRC (2001) and applied to total dry matter intake:

$$NDT (\% MS) = PBD + (2.25 \times EED) + FDNcpD + CNFD$$

The total apparent digestibility values of nutrients were calculated as the difference between ingestion and excretion in feces (Andrigueto, 1986).

Indigestible NDF (NDFi) was estimated by incubating (20 mg DM/cm^2) samples of dietary components and leftovers in a dairy crossbred male bovine with ruminal cannula for 264 hours. The bags incubated were removed and washed until complete residue removal from ruminal digestion. The remaining material was subjected to extraction with neutral and acid detergent, respectively. Potentially digestible NDF (NDFpd) was determined according to Detmann et al. (2001):

$$NDFpd = NDF - NDFi$$

Statistical analyzes

Nutrient consumption results were submitted to analysis of variance followed by linear regression adopting the level of significance of 5% using the SAS statistical program (SAS Institute Inc., Cary, NC, USA).

Results

There was no quadratic effect for any of the variables analyzed. The intake of DM, OM, CP, NDFap, and TDN did not suffer influence of the treatments evaluated ($P > 0.05$, Table 3). However, there was a linear decreasing effect of CM inclusion on intake of NFCap expressed in kg/day [NFCapI (kg/day) = 0.632 - 0.0027x; $R^2 = 0.87$] and a linear increasing effect on EE intake [EEI (kg/day) = 0.0372 + 0.0004x, $R^2 = 0.87$]. Despite the increase in EE intake, DM consumption was not compromised with an average of 1.31 kg DM/animal/day, 26.08 g/kg BW, and 69.42 g/kg $BW^{0.75}$.

We found an overall average of 2.6% of DM intake (% BW). The overall average for TDN intake was 0.88 kg/day. There was a linear decreasing effect ($P < 0.05$) for apparent digestibility of DM (DMdig = 71.7288 - 0.1035x, $R^2 = 0.92$), OM (MOdig = 73.03 - 0.1078x, $R^2 = 0.90$), NDFap (NDFap.dig = 60,9097 - 0,1189, $R^2 = 0.5197$), NFCap (NFCap.dig = 82.1212 - 0.2173x, $R^2 = 0.83$), and TDN (TDNdig = 70.5907 - 0.0905x, $R^2 = 0.92$) (Table 3) with increasing levels of Crambe meal inclusion in the diets.

Table 3 – Intake and total apparent digestibility of nutrients of lambs receiving diets with increasing levels of crambe meal (0, 25, 50, 75%) as a substitute for concentrate protein.

Variable[1]	Diet				SEM[2]	*P-value*[3]	
	0	25	50	75		Linear	Quadrático
		Intake (kg/dia)					
DM	1.36	1.39	1.31	1.18	0.056	0.256[ns]	0.479[ns]
OM	1.26	1.28	1.21	1.09	0.051	0.216[ns]	0.472[ns]
CP	0.19	0.20	0.19	0.17	0.008	0.255[ns]	0.391[ns]
EE	0.04	0.04	0.06	0.07	0.003	0.003*	0.358[ns]

NDFap	0.42	0.44	0.46	0.46	0.018	0.604^{ns}	0.647^{ns}
NFCap	0.60	0.60	0.52	0.41	0.024	0.007^{*}	0.253^{ns}
TDN	0.95	0.96	0.89	0.78	0.042	0.061^{ns}	0.264^{ns}
			Intake (g/kg de PV)				
DM	26.75	27.37	26.37	23.81	1.028	0.305^{ns}	0.453^{ns}
OM	24.74	25.20	24.31	21.82	0.948	0.276^{ns}	0.451^{ns}
CP	3.81	3.98	3.86	3.41	0.157	0.360^{ns}	0.338^{ns}
NDFap	8.33	8.72	9.22	8.95	0.144	0.377^{ns}	0.569^{ns}
			Intake (g/kg de $PV^{0.75}$)				
DM	71.39	73.08	70.01	63.20	2.722	0.277^{ns}	0.449^{ns}
OM	66.03	67.28	64.55	57.93	2.509	0.250^{ns}	0.447^{ns}
CP	10.18	10.64	10.26	9.04	0.419	0.332^{ns}	0.337^{ns}
NDFap	15.73	16.61	15.19	13.84	0.578	0.194^{ns}	0.355^{ns}
			Total apparent digestibility (%)				
DM	70.81	70.33	66.94	63.31	0.964	0.010^{*}	0.429
OM	71.96	71.74	68.03	64.21	0.969	0.008^{*}	0.371
CP	71.01	71.59	72.00	68.28	1.164	0.469^{ns}	0.373
EE	86.90	82.98	88.52	92.65	1.149	0.045^{*}	0.104
NDFap	60.03	61.64	50.21	53.93	1.532	0.049^{*}	0.736
NFCap	79.70	78.58	74.75	62.87	1.055	0.001^{*}	0.024
TDNobs.	69.85	66.07	66.8	63.07	0.921	0.017^{*}	0.437

1/ DM: Dry matter; OM: Organic mater; CP: Crude protein; EE: Ethereal extract. NDFap: Neutral detergent fiber corrected for ashes and protein; NFCap: Non-fibrous carbohydrate corrected for ashes and protein; TDNobs: Total digestible nutrients observed.
2/ SEM: Standard error of mean.
3/ * $P < 0.05$; ^{ns} non-significant ($P > 0.05$).

Discussion

Although total apparent digestibility of DM decreased with increasing levels of CM in the diets, intake was not affected (Table 3). Intake is a parameter of great importance in animal nutrition. We have found values above the minimum recommended by the NRC (2007), which is 1 kg/day of DM or 2.0% of BW for maintenance.

The palatability of CM, which is influenced by the glycosinolate content in the meal, can affect DMI (Mendonça et al. 2015). The CM used in our study was subjected to two phases of temperature treatment, one by steam injection (75ºC) and another toasting phase (105ºC). Such thermal treatments were probably capable to reduce the levels of glycosinolates to the extend that it did not influence the acceptability by the animals and, in turn, it did not affect overall DM intake.

The linear reduction ($P < 0.05$) in the NFCap intake (Table 3) observed in our study reflects the diet composition. NFCap was substantially reduced with the inclusion of CM (Table 2). The increasing linear effect ($P < 0.05$) observed for the EE intake can also be attributed to the nutritional composition of the diets, which presented increased EE content with the inclusion of soybean oil.

The levels of NDFi, ADFi, and lignin of the diets numerically increased with the inclusion of CM (Table 2), which are important factors related to digestibility inhibition. Additionally, it is suggested that the reduction in the digestibility of DM may also be related to the reduction in consumption of NFCap to the increased levels of CM (Table 2).

Canova et al. (2015) evaluated the use of CM in diets for lambs and also attributed the decrease of DM total apparent digestibility to the increasing content of ADF and lignin according to the inclusion of this by-product in the diets and, consequently, decrease of the digestibility of the fibrous fraction, similar to our findings.

In the present study, NDIN and ADIN increased with the increasing levels of CM in the diet (Table 2). According to Brás (2014), the digestibility of the generated co-product can be compromised if there is excessive heat production during the extraction of vegetable oils. Under such circumstances, the protein of the grains reacts with carbohydrates, becoming part of the ADF fraction, considerably increasing the ADIN content, which in turn is associated with decreased digestibility and, consequently, lower nutrient utilization.

However, the increase in the NDIN and ADIN fractions with the increased levels of CM were not enough to reduce the digestibility of the protein fraction (Table 3), which presented an overall average of 70.87%, indicating similar digestibility between protein sources evaluated.

The reduction of TDN (Table 3) was already expected, since it was calculated as a function of the digestibility of other nutritional components of the diet. According to Valadares et al. (1997), there is a correlation between TDN and OM digestibility, which can be inferred from the data shown. Therefore, this variable did not influence TDN intake, which for all treatments presented a value higher than recommended by the NRC (2007), which is 0.41 kg of TDN/day for lambs in maintenance.

According to Patussi et al. (2015), changes in the NDF and ADF levels of the diets are associated with the alteration of TDN contents. This fact was confirmed in our study, since increasing levels of NDF and ADF were related to reduction of NDT of the diets.

Conclusion

Crambe meal has the potential to be used as a protein source and can be used as a substitute for concentrate protein up to 75% of the crude protein of the concentrate in diets for sheep. Although the inclusion of Crambe meal had a negative effect on nutrient digestibility, total digestible nutrients were not affected, indicating a better use of energy and nitrogen available to the animals. Such results encourage further studies to develop processing techniques with the potential to improve the utilization of this co-product as animal feedstuff as well as the establishment of the best level of inclusion for each animal category.

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