Levels of inclusion of crambe meal (*Crambe abyssinica* Hochst) in sheep diet on the balance of nitrogen and ureic nitrogen in the blood serum

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Abstract. *Crambe meal, which is a co-product of biodiesel production, is a potential substitute for conventional protein sources in ruminant diets. The objective of this study was to evaluate the effect of the substitution of crude protein of the concentrate by crude protein of crambe meal with increasing levels (0, 25, 50, and 75%) on nitrogen balance and blood plasma urea nitrogen concentration in sheep. Four male sheep, rumen fistulated, were placed in metabolic crates and distributed in a 4 x 4 Latin square design. Diets were composed of 50% roughage (corn silage) and 50% concentrate. The results were submitted to analysis of variance and regression at 5% of significance. No effect was observed on nitrogen balance ($P > 0.05$). There was a linear decreasing effect ($P < 0.05$) for plasma urea nitrogen. According to the results obtained in the present study, it can be concluded that crambe meal can be used as an alternative protein feeding and it can replace up to 75% of the concentrate crude protein, since it assures consumption and use of nitrogen similar to conventional feeding.*

Keywords. *Alternative feeding, biodiesel, coproduct, crude protein.*

Introduction

The growing global concern regarding the environmental causes altogether with the search of renewable energy sources propels the use of new resources aiming at reducing the production of pollutants. The use of agroindustrial residues in animal feeding allows for reduction in feeding costs and optimizes production efficiency. In addition, it is a sustainable alternative to reuse organic matter of plant origin, while avoiding the accumulation of these residues in the environment (Brás et al. 2014).

The production of biodiesel from vegetable sources of oil generates a significant amount of coproducts that could potentially be used to feed animals, such as ethanol, glycerol, bran, and meal (Bomfim et al. 2009). Most of these coproducts are wasted because their nutritional and economic potentialities are unknown. Therefore, the study of such residues would make possible to use them as animal feed and reduce production costs while contributing to additional income generation in the biodiesel chain.

In precision farming, precisely formulating diets is a great opportunity to reduce excessive excretions of pollutants such as nitrogenous compounds, which are harmful to the environment due to their potential to pollute soil and water. Nutrient management creates a demand for nutritionists with expertise in precision farming to reduce the environmental impact of meat production chain (Klopfenstein and Erickson 2002)

Crambe (*Crambe abyssinica* Hochst) emerges as an alternative for the production of biodiesel (Roscoe et al. 2007). In addition, the co-products from oil extraction (bran and meal) have potential to replace conventional protein sources. Several studies evaluating the nutritional quality of crambe bran and meal have shown the potential of this co-product to be used in animal nutrition (Ítavo et al. 2016; Mendonça et al. 2014; Oliveira et al. 2016; Souza et al. 2015).

Therefore, the objective of this study was to evaluate the effect of the substitution of crude protein (CP) of the concentrate by CP of crambe meal (CM) at increasing levels (0, 25, 50, and 75%) on nitrogen balance (NB) and blood plasma urea nitrogen concentration 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 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) of unknown breed, castrated, and rumen fistulated 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 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 treatments consisted of different diets with increasing levels (0, 25, 50, and 75%) of

inclusion of CP from CM as a replacement for concentrate CP (Table 1). The crambe meal donated by Caramuru Alimentos S.A and used without previous treatment.

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.

Diets were balanced according to NRC (1985) for 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. Feeding was adjusted during the experimental period to allow 20% of leftovers. The roughage to concentrate ratio of the diets was approximately 50:50 based on dry matter (DM, Table 1). Corn silage as the exclusive source of roughage. Forage and concentrate were manually mixed right before feeding the animals.

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 feeding, leftovers, feces, urine, and blood

All animals were weighed on the first day of each experimental period to determine the amount of feeding as a body weight percentage (% BW). Corn silage and concentrate supplied as well as leftovers were weighed daily to estimate individual consumption (kg/day).

Leftovers were sampled every day. Diet components as well as leftovers 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.

A digestibility test was 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.

The total urine collection was performed on each animal, from the 9th to the 12th day of each experimental period. Urine produced in the 24-hour period was collected using a 10-liter plastic buckets added to 100 ml of H_2SO_4 at 20% concentration (Fonseca et al. 2006) aiming to avoid losses of nitrogen (N) compounds from the urine by volatilization and/or possible fermentation of the samples. Daily urinary production was obtained by the average production of the three consecutive days of total urine collection. From the total daily urine volume, a sample of 50 ml was taken to represent the concentrated total urine (Valadares et al. 1999), packed in polyethylene bottles, properly sealed, identified by animal and by experimental period, and stored at -20 °C for further analysis.

On the 13th day of each experimental period, blood samples were collected by puncturing the jugular vein at 00h00, 02h00, 04h00, 06h00 and 08h00 after morning feeding using VACTUBE tubes with EDTA anticoagulant solution (Diagnóstica Indústria e Comércio Ltda, Brazil). The tubes were immediately centrifuged at 2,700 rpm for 15 minutes and the plasma was frozen (-20ºC) for further quantification of urea.

Chemical analyzes and calculations

Samples of food, leftovers, and feces were analyzed to quantify DM and nitrogen according to AOAC (1997). Urine samples were thawed and homogenized to determine its total nitrogen content using Kjeldhal method (AOAC 1997).

Nitrogen balance (NB) or retained nitrogen (g/day) was the total nitrogen that was effectively retained in the animal organism, according to the equation:

$$NB = Ingested\ N - (Fecal\ N + Urine\ N)$$

Plasma urea concentration was determined using liquiform commercial kits (Labtest Diagnóstica S.A., Brazil). Blood plasma urea nitrogen was estimated as the product of urea excretion in the urine by 0.466, which corresponds to nitrogen content in urea.

Statistical analyzes

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

Results and Discussion

The ingestion and excretion of N were similar for all diets (Table 3). There was no significant effect ($P > 0.05$) or negative values for NB nor NB in relation to N ingested between the diets.

High and positive NB values suggest equilibrium between protein and dietary digestible energy (Silva et al. 2010). Tissues rapidly absorb nitrogen and excess can be excreted through the

urine. In addition to possible environmental pollution that the excretion of nitrogen might cause, animals expend energy in converting ammonia into urea, which is how they excrete nitrogen not used by the metabolism (Van Soest 1994). The data obtained for NB indicates that the consumption of CP met the requirement of the animals and that there was a retention of protein in the animal organism (Table 3).

Table 3 – Average values of ingested nitrogen, fecal nitrogen, urinary nitrogen, nitrogen balance and plasma urea nitrogen in sheep receiving diets with crude protein of concentrate replaced by increasing levels (0, 25, 50, and 75%) of crambe meal crude protein in sheep.

Variable	Diet				SEM[1]	*P-value*[2]	
	0	25	50	75		Linear	Quadratic
Ingested N	31.05	32.44	30.86	27.11	1.365	0.294^{ns}	0.363_{ns}
Fecal N	10.77	11.27	9.01	8.77	0.869	0.308^{ns}	0.835^{ns}
Urinary N	8.85	9.03	7.93	8.24	0.257	0.222^{ns}	0.901^{ns}
Nitrogen balance	11.41	12.12	13.91	10.1	1.095	0.831^{ns}	0.321^{ns}
Nitrogen balance /ingested N	0.37	0.36	0.46	0.37	0.026	0.723^{ns}	0.460^{ns}
Plasma urea nitrogen	17.58	20.53	12.49	13.95	2.046	$< 0.001^{*}$	0.383^{ns}

[1]/ SEM = Standard error of mean. ²/ * P < 0.05, ^{ns} non-significant (P > 0.05).

Assessment of NB as well as serum and urine concentration of urea provide insightful information regarding ruminant protein nutrition. Such information is important in order to avoid productive, reproductive, and environmental negative effect to an excessive provision of protein or the inadequate energy-protein synchrony in the rumen (Pessoa et al. 2009).

Plasma urea nitrogen (PUN) linearly decreased ($P < 0.05$; $PUN = 18.9764 - 0.0757x$; $R^2 = 0.45$) across different treatments evaluated (Table 3). In ruminants, PUN can be used to monitor dietary N utilization (Pessoa et al. 2009). In this sense, high PUN concentration suggests inefficiency in protein utilization and high loss of energy associated with urea synthesis from ammonia. Van Soest (1994) has established the threshold of 14 mg/dL of PUN for sheep since increased excretion of urea is observed after this value. On the other hand, Menezes et al. (2006) considers that the ideal range is 11 to 23 mg/dL of PUN. Our results were within the range found in the literature suggesting that CM supplied animals with adequate protein nutrition.

Canova et al. (2015) also did not observe a significant effect of replacing soybean meal for crambe bran on NB and PUN concentration in lambs. According to them, digestibility and metabolization of ingested nitrogen were similar between diets with soybean and crambe bran further indicating that crambe bran protein is nutritionally similar to soybean meal protein. Cruz (2015) reported a linear reduction in urinary N and PUN with increasing inclusion of crambe meal as a replacement for soybean meal. The author attributed this linear effect to improvement on usage of nitrogen by the animals, which can be influenced by nitrogen recycling. In this sense, high concentration of blood urea nitrogen suggests inefficiency in using dietary protein great energy loss.

Conclusion

Crambe meal is a potential alternative protein source for sheep. It can replace up to 75% of the concentrate crude protein, since it assures consumption and use of nitrogen similar to conventional feeding.

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