Efficiency of microbial synthesis and the flow of nitrogen compounds in sheep receiving crambe meal (*Crambe abyssinica* Hochst) replacing the concentrade crude protein

Katharine Kelly De Azevedo[1], Darcilene Maria de Figueiredo[2], Luciana Navajas Rennó[4], Raul Ribeiro Silveira[3], Roseli Aparecida Santos[2], Leandro Diego da Silva[5], Gabriel Machado Dallago[3], Juliana Aparecida Vieira[3], Gabriel Borges Pacheco[3]

[1]Master of Animal Science. [2]Animal Science Department, Federal University of Jequitinhonha and Mucuri Valleys, Diamantina, Brazil. [3]Master's student, Animal Science Department, Federal University of Jequitinhonha and Mucuri Valleys, Diamantina, Brazil. [4]Animal Science Department, Federal University of Viçosa, Viçosa, Brazil. [5]Postdoctoral fellow, Animal Science Department, Federal University of Jequitinhonha and Mucuri Valleys, Diamantina, Brazil.

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Abstract. *The objective of this study was to evaluate the effect of increasing levels (0, 25, 50, 75%) of crude protein substitution of the concentrate by crude protein of crambe meal on microbial protein synthesis and the flow of microbial nitrogen compounds in sheep. Four rumen fistulated sheep (18 months and initial average body weight of 50 kg) were distributed in a 4 x 4 Latin square design. Diets were balanced to meet the requirements for minimum gains, containing approximately 14% crude protein and 70% total digestible nutrients. The roughage to concentrate ratio was 50:50. The total urine collection was performed to determine the excretion of purine derivatives and to evaluate microbial nitrogen production and microbial protein synthesis efficiency. The results were submitted to analysis of variance and regression at 5% of significance. Even though a linear negative effect ($P < 0.05$) was observed for intake of total digestible nutrients, we have found a linear positive ($P < 0.05$) effect of crambe meal on microbial nitrogen compounds flow to the small intestine, relative intestinal nitrogen microbial flow, and efficiency of microbial protein synthesis. Thus, crambe meal can be used up to 75% of the crude protein of concentrate in diets for sheep since it improved the overall microbial efficiency.*

Keywords. *Coproduct, diet, digestibility, nitrogen balance, rumen.*

Introduction

Nutrition is associated with performance and physiology of animals. It is also the main component of the variable cost of production and the adoption of scientifically sound strategies can lead to increased efficiency in the use of inputs and productivity, contributing to decrease production cost as well as improving overall quality of the final product. The application of precision nutrition concept requires deep knowledge regarding the nutritional requirements of different animal categories and the availability of nutrients, as well as the efficiency of their use of different production processes (Branco et al. 2012).

The correct balance of diets following the "precision feeding" concept proposed by Cole et al. (2006), in which cattle nutritional management does not impair their performance, could potentially allow for the use of alternative feedstuffs that satisfactorily fulfill the nutritional requirements of the animals. Precision feeding is also a branch of precision farming and represents a great opportunity to reduce the excretion of compounds such as ammonia, which can cause negative impacts on the environment when in excess (Klopfenstein and Erickson 2002). Then, it becomes necessary to study the feasibility of including several alternative feedstuff in the diet of animals and quantifying their productivity and economic performance. One of the alternatives is to use agroindustrial by-products as animal feedstuff. However, most of these by-products have not yet been studied regarding their composition as well as economical and biological adequate inclusion levels (Lousada Júnior et al. 2006).

The production of biodiesel from vegetable sources of oil originates significant amount of coproducts that could potentially be used as animal feeding such as ethanol, glycerol, bran, and meal (Bomfim et al. 2009). The latter has great potential for use in animal feed. In addition to reduce costs and optimize production efficiency, the use of these by-products in animal nutrition serves as a sustainable alternative to reuse organic matter of vegetable origin, avoiding the accumulation of this material in the environment (Brás et al. 2014).

The protein requirements of ruminants are met by intestinal absorption of amino acids, mainly from rumen-synthesized microbial protein and rumen non-degradable protein (Valadares 1997). Microbial protein, on average, accounts for 59% of the protein that reaches the small intestine (Clark et al. 1992) indicating the importance of the study of the mechanisms of bacterial protein synthesis as well as factors related to it (Nocek and Russell 1988).

The objective of this study was to evaluate the effect of crude protein substitution (CP) from concentrate to CP of crambe meal (CM) on the microbial protein synthesis and the flow of microbial nitrogen compounds from the rumen in lambs.

Materials and Methods

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).

Four male lambs (18 months old and initial body weight of 50 kg) of unknown breed and

castrated, were selected from the herd and fistulated in the rumen according to the procedure described by Gomes et al. (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.

Diets were balanced according to NRC (2007) 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.

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.

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 compounds from the urine by volatilization and/or possible fermentation of the samples. Sieve was placed at the end of the urine collector tube of the crates to avoid possible contamination (feces, hairs, etc.) of the urine.

The 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 and another sample of 10 mL was diluted in 40 mL of H_2SO_4 0.036 N to represent total diluted urine (Valadares et al. 1999). Both were packed in polyethylene bottles, properly sealed, identified by animal and by experimental period, and stored at -20 °C for further analysis. Total N excretion was measured using concentrated

samples. Urea and purine derivatives were determined using diluted urine samples.

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). 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):

$$NDFpd = NDF - NDF_i$$

Absorbed microbial purines (Pabs) were calculated based on the excretion of total purine derivatives (TPD) in the urine using the equation proposed by Chen and Gomes (1992) for sheep:

$$DPT = 0.84Pabs + 0.150\ BW^{0.75}\ e^{-0.25Pabs}$$

where 0.84Pabs = absorption efficiency of exogenous purines, 0.150 BW$^{0.75}$ = endogenous excretion of purine derivatives relative to the metabolic body weight of the animal, and $e^{-0.25Pabs}$ = substitution rate by *de novo* synthesis of endogenous purines. Pabs were estimated by solving the equation using the Newton-Raphson interaction process (Chen and Gomes 1992). The intestinal flow of microbial N (NMIC) was estimated based on the amount of Pabs (mmol / day), according to the equation described by Chen and Gomes (1992):

$$NMIC\ (g/day) = \frac{Pabs\ (mmol/day) \times 70 = 0{,}727Pabs}{0{,}83 \times 0{,}116 \times 1000}$$

Assuming the digestibility of 0.83 for microbial purines, the ratio 0.116 of N purine to total N and N content of purines of 70 mg N/mmol. The efficiency of microbial protein synthesis (EFIM) was expressed in g of microbial CP/100 g of total digestible nutrients consumed (g of CPmic/100 g of TDN).

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

Results

Even though a linear negative effect ($P < 0.05$) was observed for observed TDN (NDTobs = 70.5907 –0,0905x; R^2 = 0,92), we have found a linear positive ($P < 0.05$) effect of CM on microbial nitrogen compounds flow (NMIC = 10.0237 + 0.0309x; R^2 = 0.91) to the small intestine in sheep (Table 3). The same effect ($P < 0.05$) was observed for NMIC, which represents the NMIC in relation to the nitrogen ingested (NMICR = 0.3071 + 0.0015x; R^2 = 0.75) and efficiency of microbial synthesis (EFIM = 6.1774 + 0.0986x; R^2 = 0.85; Table 3).

Table 3 – Microbial nitrogen synthesis, intake, and apparent digestibility of total digestible nutrients as a function of increasing levels of crambe meal in diets fed to sheep.

Variable[1]	Diet				SEM[2]	P-value[3]	
	0	25	50	75		Linear	Quadratic
Total digestible nutrient observed	69.85	66.07	66.80	63.07	0.921	0.017*	0.437ns
Nitrogen intake	31.05	32.44	30.86	27.11	1.365	0.294ns	0.363ns
Microbial nitrogen synthesis							
NMIC (g/day)	10.17	10.78	11.14	12.63	0.347	0.027*	0.540ns
NMICR (g/g de N intake)	0.33	0.33	0.35	0.44	0.017	0.037*	0.205ns
EFIM (g CP/100g TDN)	7.15	7.91	10.19	14.69	1.211	0.023*	0.362ns

[1]/ NMIC – intestinal flow of microbial nitrogen, NMICR – relative intestinal nitrogen microbial flow, EFIM – Efficiency of microbial crude protein synthesis. [2]/ SEM – Standard error of mean. [3]/ * $P < 0.05$, [ns] non-significant ($P > 0.05$).

Discussion

In order to maximize microbial growth, it is necessary to synchronize energy supply with degradable nitrogen in the rumen (Lima et al. 2013). Therefore, diets with reduced supply of non-fibrous carbohydrates (Table 2) could result in reduction of microbial nitrogen flow to the small. However, we did not find such results in our study (Table 3), suggesting that CM improved amino acids and N-NH_3 assimilation by ruminal microorganisms. In addition, the reduction in TDN intake (Table 3) might have not been great enough to limit microbial growth.

Crambe meal is rich in amino acids such as cysteine, methionine, lysine, and threonine (Goes et al. 2010). Thus, the variation of some essential amino acids between experimental diets may have contributed to greater microbial synthesis with increasing levels of crambe meal.

The overall average EFIM in our study was 10.92 g PBmic/100 g NDT is similar to previous studies with small ruminants receiving diets with nutritional composition similar to the present study. Literature results range from 5.8 to 12.02 g PBmic/100 g NDT (Carvalho et al. 2010; Fonseca et al. 2006; Freire et al. 2012).

Conclusion

Crambe meal has the potential to be used as a protein source and it can substitute up to 75% of the crude protein of concentrate in diets for sheep. The inclusion of PB from crambe meal improved the intestinal flow of microbial nitrogen and the overall efficiency of microbial protein synthesis.

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References

AOAC (1997). *Official methods of analysis of AOAC International* (16 ed. ed., Official methods of analysis of AOAC International.). Gaithersburg, MD: AOAC International.

Bomfim, M. A. D., Silva, M. M. C., & Santos, S. F. (2009). Potencialidades da utilização de subprodutos da indústria de biodiesel na alimentação de caprinos e ovinos. *Tecnologia e Ciência Agropecuária, 3*, 15-26.

Branco, A. F., D.L., H., & Osmari, M. P. (2012). *Nutrição de precisão aplicada a nutrição de ruminantes.* Paper presented at the 49º Reunião Anual da Sociedade Brasileira de Zootecnia, Brasília.

Brás, P., Possenti, R. A., Bueno, M. S., Canova, E. B., & Schammas, E. A. (2014). Avaliação nutricional de coprodutos da extração de óleos vegetais em dieta de ovinos. *Boletim de Indústria Animal, 71*(2), 16, doi:10.17523/bia.v71n2p160.

Carvalho, G. G. P. d., Garcia, R., Pires, A. J. V., Silva, R. R., Pereira, M. L. A., Viana, P. T., et al. (2010). Balanço de nitrogênio, concentrações de ureia e síntese de proteína microbiana em caprinos alimentados com dietas contendo cana-de-açúcar tratada com óxido de cálcio. *Revista Brasileira de Zootecnia, 39*, 2253-2261.

Chen, X. B., & Gomes, M. (1992). *Estimation of microbial protein supply to sheep and cattle based on urinary excretion of purine derivatives-an overview of the technical details*. Aberdeen: International Feed Resources Unit / Rowett Research Institute.

Clark, J. H., Klusmeyer, T. H., & Cameron, M. R. (1992). Microbial Protein Synthesis and Flows of Nitrogen Fractions to the Duodenum of Dairy Cows1. *Journal of Dairy Science, 75*(8), 2304-2323, doi:10.3168/jds.S0022-0302(92)77992-2.

Cole, N. A., Defoor, P. J., Galyean, M. L., Duff, G. C., & Gleghorn, J. F. (2006). Effects of phase-feeding of crude protein on performance, carcass characteristics, serum urea nitrogen concentrations, and manure nitrogen of finishing beef

steers1,2. *Journal of Animal Science, 84*(12), 3421-3432, doi:10.2527/jas.2006-150.

Detmann, E., Cecon, P. R., Paulino, M. F., Zervoudakis, J. T., Valadares Filho, S. d. C., & Araújo, C. V. d. (2001). Estimação de parâmetros da cinética de trânsito de partículas em bovinos sob pastejo por diferentes seqüências amostrais. *Revista Brasileira de Zootecnia, 30*, 222-230.

Fonseca, C. E. M., Valadares, R. F. D., Valadares Filho, S. d. C., Leão, M. I., Cecon, P. R., Rodrigues, M. T., et al. (2006). Estimativa da produção microbiana em cabras lactantes alimentadas com diferentes teores de proteína na dieta. *Revista Brasileira de Zootecnia, 35*, 1169-1177.

Freire, L. D. R., Moreira, B. S., Pereira, M. M., Ferreira, D. N., dos Santos, E. d. J., dos Santos Pedreira, M., et al. (2012). Uréia de Liberação Lenta em Dietas de Ovinos Confinados: Síntese de Proteína Microbiana. *Revista Científica de Produção Animal, 14*(2), 219-222, doi:10.15528/2176-4158/rcpa.v14n2p219-222.

Goes, R. H. T. e. B., Alves de Souza, K., Patussi, R. A., Cornelio, T. C., Oliveira, E. R., & Brabes, K. C. S. (2010). Degradabilidade in situ dos grãos de crambe, girassol e soja, e de seus coprodutos em ovinos. *Acta Scientiarum. Animal Sciences, 32*(3), 271-277.

Gomes, G. M. F., Egito, A. S., Salles, H. O., Oliveira, E. L., & Vasconcelos, A. M. (2009). *Seleção de animais e cuidados pré, durante e pós-cirúrgicos na fistulação ruminal em caprinos e ovinos*. Sobral, Ceará, Brazil: Embrapa Caprinos e Ovinos.

Klopfenstein, T. J., & Erickson, G. E. (2002). Effects of manipulating protein and phosphorus nutrition of feedlot cattle on nutrient management and the environment. *Journal of Animal Science, 80*(E. Suppl. 2), E106–E114.

Lima, H. L., Goes, R. H. T. B. D., Cerilo, S. L. N., Oliveira, E. R. D., Gressler, M. G. M., & Brabes, K. C. S. (2013). Nutritional parameters of steers receiving different levels of sunflower crushed in partial replacement of soybean meal. *Anais da Academia Brasileira de Ciências, 85*, 1513-1522.

Lousada Júnior, J. E., Costa, J. M. C., Neiva, J. N. M., & Rodriguez, N. M. (2006). Caracterização físico-química de subprodutos obtidos do processamento de frutas tropicais visando seu aproveitamento na alimentação animal. *Revista Ciência Agronômica, 37*(1), 70-76.

Mertens, D. R. (2002). Gravimetric determination of amylase-treated neutral detergent fiber in feeds with refluxing in beakers or crucibles: collaborative study. *Journal of AOAC International, 85*(6), 1217-1240.

Nocek, J. E., & Russell, J. B. (1988). Protein and Energy as an Integrated System. Relationship of Ruminal Protein and Carbohydrate Availability to Microbial Synthesis and Milk Production. *Journal of Dairy Science, 71*(8), 2070-2107, doi:10.3168/jds.S0022-0302(88)79782-9.

NRC (2007). *Nutrient requirements of small ruminants: sheep, goats, cervids, and new world camelids*. Washington, DC: The National Academies Press.

Valadares, R. F. D. (1997). *Níveis de proteína em dieta para bovinos: consumo, digestibilidade, eficiência de síntese microbiana, amônia ruminal, ureia plasmática, e excreções de uréia e creatinina*. Thesys, Universidade Federal de Minas Gerais, Belo Horizonte, Minas Gerais, Brazil.

Valadares, R. F. D., Broderick, G. A., Valadares Filho, S. C., & Clayton, M. K. (1999). Effect of Replacing Alfalfa Silage with High Moisture Corn on Ruminal Protein Synthesis Estimated from Excretion of Total Purine Derivatives1. *Journal of Dairy Science, 82*(12), 2686-2696, doi:10.3168/jds.S0022-0302(99)75525-6.