

Degradability and intestinal digestibility of protein in diets containing different levels of cashew types

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Abstract:

The objectives of this study to estimate the rumen degraded protein (RDP) and intestinal digestibility of rumen undegraded protein (IDRUP) of seven different diets containing two levels of cashew types of early dwarf cashew type (CCP06 and BRS189), using the in vitro techniques and three steps protein digestibility. The substrates were incubated in vitro for 16 hours to determine the RDP. The residues were digested with pepsin solution for 1 hour, and pancreatic solution at 39°C for 24 hours. Then, these residues were analyzed for crude protein. We adopted a randomized block design with seven treatments and three replications. The data were subjected to analysis of variance and the means bought by SNK test ($\alpha = 0.05$). The control diet and diet containing 150 g/kg of cashew CCP06 gave the highest values ($P < 0.05$) rumen degradable protein (RDP). However, no significant difference ($P > 0.05$) between the diets, the coefficient of rumen degradable protein was 0.661 ± 2.7 g / kg. The diets did not differ ($P > 0.05$) for protein undegraded in the rumen and digestible in the small intestine. The control diet and diet containing 150 g/kg of cashew CCP06 were the most promising because they have higher levels of rumen degradable protein. The diets had intestinal digestibility of rumen undegraded protein constant.

Keywords: Rumen degradable protein, rumen undegradable protein, digestible protein in the intestine

Introduction

The protein diet degradation in the rumen has great influence on the supply of amino acids for ruminants. This consists of two fractions: rumen degradable protein (RDP) and rumen undegradable protein (RUP). The PDR gives rise to peptides, amino acids and ammonia, and is used by rumen microorganisms for microbial protein synthesis, which is typically the major source of protein to ruminants. While RUP is the second source of amino acids for the animal, represented by dietary protein that escapes from ruminal fermentation. By presenting amino acid composition and intestinal digestibility variable it is necessary to accurately estimate their intestinal digestibility. Knowledge of RDP and RUP give a great importance to assess the quality and quantity of protein available to rumen microorganisms and ruminants, it allows changing the ingredients of rations in order to maximize productivity and minimize costs.

The cashew culture is one of great socio-economic importance for the Northeast region of Brazil, responsible for 100% of economic exploitation, concentrated mainly in the states of Ceará, Rio Grande do Norte and Piauí (IBGE, 2010). The main objective of the operation of cashew has been obtaining Chestnut, whose almond processing results. This practice results in large quantities of stem that are not utilized, the field is abandoned. However, knowledge about the use of this crop for animal feed is still quite empirical.

In this sense, this study aimed to estimate the (RDP) and intestinal digestibility of rumen undegraded protein (IDRUP) of seven diets without adding pseudo cashew (control diet) and with the inclusion of three levels (150, 300 and 450 g / kg) of cashew clones of two precocious dwarf cashew type (CCP06 and BRS189), using *in vitro* three steps techniques.

Materials and Methods

This study was conducted in the Laboratory of Animal Nutrition, the Center for Nuclear Energy in Agriculture, University of São Paulo (LANA-CENA/USP) in Piracicaba, SP. Seven diets have been evaluated: no addition of pseudo cashew (control diet) and with the inclusion of three levels (150, 300 and 450 g / kg) of two clones of cashew cashew-type dwarf early CCP06 and BRS189 (Table 1). The pseudo cashew clones were obtained from clonal Embrapa Tropical Experiment Station Pacajus, Ceará. The cashew underwent pre-drying in the sun for 20 days, to remove some of the moisture, and later taken to the stove with forced air at 55 °C for 72 hours. They were then ground in a mill with a sieve of 1 mm sieves and stored in sealed containers. Diets were formulated to meet the nutritional requirements of sheep with 30 kg body weight and average daily gain of 200 g according to (NRC, 2007).

The chemical composition of the samples (Table 2) was obtained by AOAC (1995) for dry matter (DM), organic matter (OM), ether extract (EE) and crude protein (CP). Neutral detergent fiber (NDF), acid detergent fiber (ADF) and acid detergent lignin (ADL)

were determined according to Van Soest et al. (1991), performed sequentially.

In order to estimate the (RDP), we used the methodology described by Calsamiglia and Stern (1995), with adaptations. Incubation was performed in an incubator *in vitro* model TE-150 (Tecnal), using three different inoculums, each from two donors. The samples, approximately 2 g, were placed in nylon bags previously weighed and identified, divided into three major nylon bags, one for each type of inoculum. The samples were incubated for 16 hours at 39 °C, with 1,300 ml of nutrient medium, 650 mL of ruminal and CO₂. After this period, bags were washed in running water for about 2 hours and subsequently in a washing machine for 1.5 hours, changing the water every 15 minutes. Then were frozen at -10 °C overnight and washed again for 30 minutes. After drying in an oven with forced air at 50 °C for 48 hours, were weighed. The waste used to determined crude protein concentrations.

Table 1. Feed composition of experimental diets, as dry matter basis

Ingredientes (g/kg)	Diets						
	Control diet	CCP06 (g/kg)			BRS189 (g/kg)		
		150	300	450	150	300	450
Tifton hay 85	500	480	460	410	500	500	400
Corn	300	200	60	-	180	30	-
Soya bean meal	150	150	160	120	150	150	130
Premix	20	20	20	20	20	20	20
Pseudofibro CCP06	-	150	300	450	-	-	-
Pseudofibro BRS189	-	-	-	-	150	300	450

* Levels of product per kg: Vitamin A 160 000 IU Vitamin D3 40,000 IU Vitamin E 520 IU 210 grams calcium, phosphorous 6 g, 75 g sodium, chloride 180 g, 180 mg manganese, 180 mg zinc, iron 740mg, cobalt 10 mg, 24 mg iodine and selenium 2.2 mg.

Table 2. Chemical composition of sheep diets formulated with cashew clones (*Anacardium occidentale* L.)

ADL	152,0	114,0	21,7	53,0	90,1	97,2	44,1	47,7	60,9
(g/kg)	CCP06	BRS189	Control diet	DCCP06 (g/kg)			DBRS189 (g/kg)		
				150	300	450	150	300	450
DM	892,9	850,3	885,5	885,4	887,2	888,6	879,3	878,7	882,1
OM	962,0	963,5	936,5	930,2	931,2	929,4	929,4	929,4	932,8
EE	52,1	25,6	22,5	24,5	27,0	29,4	22,9	19,6	20,7
CP	114,2	81,3	148,3	147,4	142,1	135,2	134,1	123,8	118,9
NDF	374,4	269,1	543,6	543,1	507,6	543,4	547,1	570,2	427,4
ADF	281,8	183,5	243,9	280,7	286,2	326,0	275,6	288,2	253,9

¹ DM = dry matter, OM = organic matter, EE = ether extract, CP = crude protein, NDF = neutral detergent fiber, ADF = acid detergent fiber, ADL = acid detergent lignin

The estimate of *in vitro* intestinal digestibility of RUP, or, the and intestinal digestibility of rumen undegraded protein (IDRUP), was obtained by the technique of three steps, as Calsamiglia and Stern (1995). Incubated 0.5 g of residue after 16 h incubation *in vitro* with 10 mL of 0.1 N HCl containing 1 g / l of pepsin (pH = 1.9) for 1 hour at 39 ° C. Subsequently were added 0.5 ml of 1 N NaOH for neutralization of pH and 13.5 ml of pancreatin solution (0.5 M KH₂PO₄ solution, pH = 7.8) containing 50 ppm of thymol, for prevent microbial growth, and 3 g / L pancreatin for 24 hours at 39 ° C. Then was added 3 mL of tri-chloro acetic acid (TCA) to 100%, for 15 minutes to precipitate the protein. Centrifugation was performed at 9500 rpm for 15 minutes and the supernatant quantitated soluble protein content (AOAC, 1995). The IDP was calculated from the PB digested after incubation with pepsin and pancreatin incubated and protein.

We adopted the experimental design in blocks with seven treatments (diets) and three replications (inoculants). Data were subjected to analysis of variance by Generalized Linear Model procedure (PROC GLM) of SAS (Statistical Analysis System, version 8), and averages bought by SNK test. $\alpha = 0.05$.

Results and Discussion

Using the control diet and the diet containing 150 g / kg of cashew clones CCP06 gave higher values ($P < 0.05$) for rumen degradable protein (RDP), revealing himself as the best source of protein to rumen microorganisms in comparison with the other diets (Table 3). However, to make best use of this RDP there should be availability energy sources of rapid degradation in the rumen.

Table 3. Crude protein (CP) and coefficients of degradability and intestinal digestibility *in vitro* of protein diets which containing pseudo clones of cashew (*Anacardium occidentale* L.)

Parameters ^a	Diets									LPM ¹
	Control Diet	DCCP06 (g/kg)			DBRS189 (g/kg)					
		150	300	450	150	300	450			
CP (g/kg of DM)	148.3	147.4	142.1	135.2	134.1	123.8	118.9			
RDP (g/kg of CP)	513.7 ^a	542.2 ^a	377.6 ^b	323.8 ^b	432.4 ^b	302.5 ^c	332.5 ^c		36	
IDP (g/kg of RUP)	774.3	731.9	638.3	572.6	709.1	629.5	569.1		12	
IDRUP (g/kg of DM)	0.558	0.494	0.564	0.524	0.540	0.544	0.451		6.7	

RDP = rumen degraded protein; RUP = rumen undegraded protein, 1 DP= the intestine digestible protein in; IDRUP = protein not degraded

in the rumen and digestible in the intestine. ²EPM = standard error of the mean. Means in row followed by different letters are different by SNK test ($P < 0.05$).

Diets containing 300 g / kg and 450 g / kg of cashew clones CCP06 BRS189 had lower ($P < 0.05$) values of RDP in relation to the control diet and the diet containing 150 g / kg of cashew clones CCP06, indicating lower availability of protein to microbial attack. Thus, the higher amount of protein to reach the duodenum intestinal digestion can be used as a source of amino acids for the animal. The supply of adequate amounts of RDP and RUP is critical to optimize the production of microbial protein and amino acid supplement the requirements of ruminants, respectively (Santos, 2006). As for the coefficient of rumen degradable protein (IDP), no difference ($P > 0.05$) among diets, averaging 0.661 ± 2.7 g / kg. This protein is principally derived from the fraction of slow degradation in the rumen digestibility of which ranged between 50 and 80% (Sniffen et al. 1992). The diets did not differ ($P > 0.05$) for rumen undegraded protein and digestible in the small intestine (IDRUP). These values, provide an index to assess the quality of foods as sources of RUP for ruminants.

Conclusions

All diets had intestinal digestibility of rumen undegraded protein constant. However, the control diet and the diet containing 150 g / kg of cashew clones CCP06 were the most promising because they have higher levels of rumen degradable protein.

Literature cited

- ASSOCIATION OF OFFICIAL ANALYTICAL CHEMISTS - AOAC. **Official methods of analysis**. 16.ed. Washington: AOAC, 1995. 2000p.
- CALSAMIGLIA, S.; STERN, M.D. A three-step *in vitro* procedure for estimating intestinal digestion of protein in ruminants. **Journal of Animal Science**, v.73, p.1459-1465, 1995.
- INSTITUTO BRASILEIRO DE GEOGRAFIA E ESTATÍSTICA - IBGE. Anuário estatístico do Brasil. Disponível em: <<http://www.sidra.ibge.gov.br/bda/tabela/listabl.asp?c=1618>>, Acesso em: 23/12/2010.
- NATIONAL RESEARCH COUNCIL - NRC. **Nutrients requirements of small ruminants: sheep, goats, cervids, and new world camelids**. Washington: National Academy Press, 2007. 362p.

SANTOS, F.A.P. Metabolismo de proteínas. In: BERCHIELLI, T.T.; PIRES, A.V.; OLIVEIRA, S.G. (Eds) **Nutrição de ruminantes**. 1.ed. Jaboticabal: Funep, 2006. p.255-286.

SNIFFEN, C.J.; O'CONNOR, J.D.; VAN SOEST, P.J. et al A net carbohydrate and optimal protein system for evaluating cattle diets. II. Carbohydrate and protein availability. **Journal of Animal Science**, v.70, p.3562-3577, 1992.

VAN SOEST, P.J.; ROBERTSON, J.B.; LEWIS, B.A. Methods for dietary fiber, neutral detergent fiber, and nonstarch polysaccharides in relation to animal nutrition. **Journal of Dairy Science**, v.74, n.10, p.3583-3597, 1991.