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Tannin on non-degradable digestible protein from proteic sources in cattle rumen

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ABSTRACT. Several tannins at different inclusion levels of protein-rich food and water addition on the amount of rumen undegradable protein (RUP) and digestible RUP (RUPd) in the rumen were evaluated. Sixty treatments were analyzed, namely: three mixtures of tannin (with different concentrations of hydrolysable and condensed tannins) were added at four different amounts (0, 1, 2.5 and 5%) in three protein foods (soybean meal, whole soybean meal and peanut meal) with and without moisture. Samples were incubated in cattle, via rumen cannula, in triplicate, to quantify rumen degraded protein (RDP), rumen undegradable protein (RUP) and digestible RUP (RUPd). Divergence in protein nutritional rate, based on discriminating variables among the groups, was estimated by cluster analysis. Increase in RUPd of treatments required soybean meal with 2.5% tannin, with 85% of condensed tannins and 15% hydrolysable tannins, in an aqueous medium. The inclusion of tannin is recommended to test in *in vivo* evaluations for productivity increase and inclusion level used.

Keywords: degradation, protein efficiency, rumen fermentation.

Utilização do tanino sobre a proteína digestível não degradada no rúmen de alimentos protéicos em bovinos

RESUMO. Este trabalho foi realizado para avaliar os tipos de taninos sob diferentes níveis de inclusão em alimentos protéicos com adição de água, sobre a quantidade de proteína não degradável no rúmen (PNDR) e PNDR digestível (PNDRd). Foram analisados 60 tratamentos, arranjados da seguinte forma: adição de três misturas de tanino (com diferentes concentrações de taninos hidrolisáveis e condensados) que foram adicionados em quatro diferentes quantidades (0; 1; 2,5 e 5%) sob três alimentos protéicos (farelo de soja, farelo de soja integral e farelo de amendoim) que passaram ou não por processo de umedecimento. As amostras foram incubadas em bovinos, via cânula ruminal, em triplicata, para a quantificação da proteína degradada no rúmen, PNDR e PNDRd. A divergência do valor nutricional proteico, baseada em variáveis discriminatórias entre os grupos, foi estimada por meio de análise de agrupamento. Para aumentar o teor de PNDR digestível da dieta recomenda-se a utilização de farelo de soja tratado, em meio aquoso, com 2,5% de tanino com 85% de tanino condensado e 15% de tanino hidrolisável. Recomenda-se testar a inclusão desse ingrediente em avaliações *in vivo* para determinar o aumento de produtividade e o nível de inclusão a ser adotado.

Palavras-chave: degradação, eficiência protéica, fermentação ruminal

Introduction

Most protein ingested by cattle undergoes rumen degradation due to the intense fermentation capacity of rumen bacteria. On certain occasions, however, especially in intense production systems, degradation is reduced to increase rumen undegradable protein (RUP). Strategies for protein protection are employed in determined situations to minimize rumen fermentation. They decrease rumen degradation and make available amino acids for intestinal absorption to protect high biologically rated proteins from rumen fermentation. Tannins are an important alternative for this role (Archana, Jadhav, & Kadam, 2010; Benchaar, McAllister & Chouinard, 2008; Khiaosa-Ard et al., 2009).

Tannins are polymers that complex with proteins and decrease the degradation in the rumen (Mezzomo et al., 2011). Their activities mainly occur by hydrogen and hydrophobic bonds, where the latter is effective only in a water medium. Further, tannins may be condensed and hydrolyzed. Although, as a rule, hydrolysable tannins have a greater capacity to bond with proteins, they may be degraded by chemical or enzymatic hydrolysis in several structural units that compose them, by decreasing or eliminating the capacity of bonding with protein (Khanbabaee & Van

Ree, 2001). On the other hand, condensed tannins are not degraded by natural enzyme processes and their high molecular weight decreases their bonding capacity with proteins when compared to hydrolysable tannins (Archana et al., 2010).

Current research evaluates types of tannin at different inclusion levels in protein food, with the addition of water, on the amount of RUP and of digestible RUP (RUPd).

Material and methods

The experiment was conducted at the Animal Laboratory of the Department of Animal Science of the Federal University of Viçosa, Viçosa, Minas Gerais State, Brazil. Three protein diets were employed: soybean meal (SM), whole soybean meal (SMw) and peanut meal (PM). SMw is composed of roasted soybean meal with the lowest oil extraction and approximately 8% ether extract. Table 1 gives the chemical composition of the meals.

Three different commercial mixtures of tannins (A, B and C) at 0, 1, 2.5 and 5% of each mixture were added to meals. Tested tannin mixtures had different proportions of condensed tannin:hydrolysable tannin, namely, 85:15 (mixture A), 55:45 (mixture B) and 20:80 (mixture C). Quebracho extract (*Schinopsis* sp.) was the source of condensed tannin, whilst chestnut (*Castanea sativa*) and tara (*Caesalpinia spinosa*) were the source of hydrolysable tannin.

Table 1. Chemical composition of meals.

Meals	DM (%)	Item[1]					
		OM	CP	EE	NDFcp	ADF	NFC
Soybean meal	88.27	93.45	43.81	1.33	13.77	8.52	34.54
Whole soybean meal	90.10	95.47	43.50	8.64	10.44	9.21	32.89
Peanut meal	92.16	95.67	52.02	0.53	11.46	9.88	31.66

[1]% of dry matter (DM); OM: organic matter; CP: crude protein; EE: ether extract; FDNcp: neutral detergent fiber corrected for ashes and proteins; CNF: total non-fibrous carbohydrates; FDA: acid detergent fiber.

After adding the tannin, 50 g of each food sample were separated and solubilized with 100 mL of water at pH 5.5, with 1:2 weight:volume, and left to rest for 6 hours. The samples were then dried in a forced-air buffer at 55ºC for 72h. Further, the samples (treated or not with water) were processed in a Wiley mil (1 and 2 mm) and stored in polyethylene flasks for analysis.

Fifty-four treatments were analyzed (in triplicate) by combining three protein meals, three tannin mixtures, three levels of tannin mixtures, with moisture or not, of the sample (Figure 1). Control was the moist meal or not, without tannin, with six more treatments.

Each treatment is given as *XX–U T W*%
where:

"XX": is the mixture´s ingredient, represented by SM, SMw or PM, respectively for soybean meal, whole soybean meal and peanut meal;

"U" is the treatment with water (lack of U shows that treatment was not submitted to solubilization);

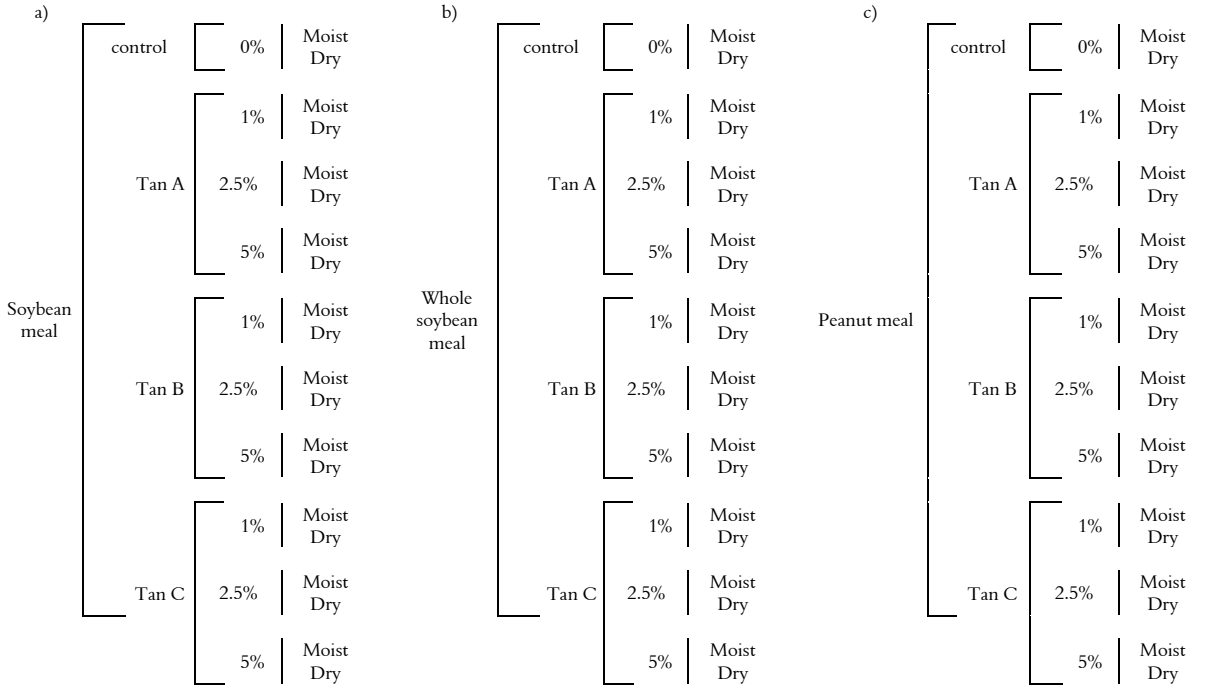

Figure 1. Scheme of meal treatments.

"T" is the type of tannin, for mixtures A, B or C, according to above;

"W%" is the amount of TC in the sample: 0, 1, 2.5 or 5%.

Samples, processed at 2 mm, were incubated *in situ*, in triplicate, to quantify the rumen degradable protein (RDP) and RUP. Three crossbred bulls, fed on a diet with 65% concentrate composed of ground corn, soybean meal, urea and mineral nucleus, and with 35% corn silage, were used.

Intestine digestibility of the protein was assessed according to Calsamiglia & Stern (1995). Samples were placed in 44 μ nylon bags (20-25 mg of sample/cm^2) and incubated *in situ* for 16 hours. After rumen incubation, the bags were washed in running water till total whitening. They were then placed in a forced air buffer at 60ºC for 72 hours and nitrogen in the residue was quantified following method INCT-CA N-001/1 (Detmann et al., 2012). Aliquots of the incubation residue with approximately 15 mg of N were placed in 50 mL centrifuge tubes. The tubes were incubated with 10 mL of solution 0.1 N of HCl, with 1 g L^{-1} of pepsin (pH = 1.9) and stirred at 40 rpm, during 1 hour, at 38ºC. Further, 0.5 mL of the solution 1 N of NaOH were added to neutralize acidity, and 13.5 mL of pancreatin solution with 0.5 M of the solution KH_2PO_4 (pH = 7.8), 50 ppm thymol and 3 g L^{-1} pancreatin. The samples were stirred at 40 rpm for 24 hours, at 38ºC. At the end of digestion, 3 mL of trichloroacetic acid (TCA) 100% (weight/volume) solution were added to stop enzyme activity and precipitate the non-digested proteins. The samples were then centrifuged for 15 min. at 10,000 x g and the supernatant was used to evaluate residual N, following method INCT-CA N-001/1 (Detmann et al., 2012). Intestinal digestibility of NDPR was calculated as the ratio between the amount of digested CP after incubation with pepsin and the quantity of protein incubated *in vitro*.

RDP was quantified by the difference between total incubated protein and residual protein in the bag after 16h incubation. However, RUP was given as total residual protein due to total incubated protein. RUPd was calculated by multiplying RUP by *in vitro* intestinal digestibility.

Divergence of the nutritional protein rate, based on discriminatory variables among the groups (variables: rumen degraded protein and digestible rumen undegradable protein in the rumen) was calculated by cluster analysis, employing mean Euclidian distance with standardized variables and the optimization clustering method (Tocher´s method). The System of Statistical and Genetic Analyses (SAEG) was employed for statistical analyses.

Results and discussion

Tables 2, 3 and 4 show results of RDP, intestinal digestibility of RUP and RUPd, respectively, according to experimental treatments.

Table 2. Degradable crude protein in the rumen (RUP, %) of three protein meals, moist (U) or not, treated with tannin mixtures (A, B and C) and inclusion levels of each meal (1, 2.5 and 5%).

Item[1]		Level of tannin mixture			
		0%	1%	2.5%	5%
SM		74.01±2.08			
	A	-	69.65±3.58	70.98±3.22	70.68±1.04
	B	-	73.86±3.63	71.40±2.48	66.45±0.48
	C	-	71.95±1.89	69.87±7.56	66.28±3.05
SM - U		64.38±7.57			
	A	-	36.87±3.29	23.22±2.09	22.53±1.67
	B	-	31.24±2.54	30.70±2.45	21.29±2.77
	C	-	33.01±3.39	32.19±2.61	22.05±1.25
SMw		56.20±2.74			
	A	-	56.37±2.27	53.81±2.60	52.95±2.46
	B	-	52.94±2.07	52.20±1.29	51.60±3.96
	C	-	54.71±3.75	50.46±2.53	51.58±3.54
SMw - U		55.12±4.17			
	A	-	49.60±3.92	45.83±2.53	38.94±2.36
	B	-	46.25±1.28	43.84±2.86	42.61±1.22
	C	-	46.13±4.51	44.69±2.03	42.58±2.70
PM		71.14±6.65			
	A	-	64.53±4.96	56.17±1.73	50.38±3.70
	B	-	60.02±4.04	57.58±3.46	50.62±3.92
	C	-	53.91±2.40	54.35±3.54	50.15±1.99
PM - U		54.24±6.44			
	A	-	38.00±1.82	37.77±1.33	41.33±5.96
	B	-	44.26±2.19	39.14±2.09	31.12±3.48
	C	-	46.65±3.06	41.54±2.77	37.40±2.50

[1]SM = soybean meal; SMw = whole soybean meal; PM = peanut meal. A, B and C = mixtures of tannin with different ratios of condensed tannin/hydrolysable tannin (CT/HT), respectively 85/15; 55/45; 20/80.

Rumen degradable protein (RDP) of all meals tested decreased when meal plus tannin was moistened. Highest decrease rates were reported for soybean meal with up to 65.75% of RDP. Reduction for peanut meal was less, up to 42.65%. Table 3 shows that there were no great changes in intestinal digestibility of RUP when tannins were used in the food protein complex. In fact, bonds between tannin and the protein in meals did not change the activities of protein-digesting enzymes. The result was expected since tannins do not maintain the complex tannin x protein in an acid medium. Lack of alterations in the intestinal digestibility of RUP and increase in RUP amounts (when tannin was used) raised RUPd in the meals (Table 4).

Table 3. Intestinal digestibility of rumen undegradable protein (RUP; %) of three protein meals, moistened (U) or not, treated with tannin mixtures (A, B and C) and inclusion levels in each meal (1, 2.5 and 5%).

Item[1]		Level of tannin mixture			
		0%	1%	2.5%	5%
SM		73.67±1.94			
	A	-	74.70±1.06	74.25±1.62	71.75±2.03
	B	-	75.04±1.29	75.81±1.58	72.40±1.19
	C	-	74.25±2.08	71.02±1.10	70.04±0.68
SM – U		52.32±0.40			
	A	-	76.06±2.49	75.60±2.17	67.92±1.14
	B	-	72.38±2.31	68.70±1.60	67.13±1.92
	C	-	67.36±2.89	65.35±1.97	67.06±2.61
SMw		79.23±0.91			
	A	-	80.62±1.27	78.20±0.69	80.38±1.01
	B	-	75.32±1.12	74.25±1.08	75.60±1.78
	C	-	77.29±1.02	78.47±1.33	78.13±2.13
SMw – U		72.17±1.89			
	A	-	74.57±1.96	70.73±1.15	73.19±1.35
	B	-	72.24±1.41	69.43±1.80	77.84±0.72
	C	-	72.64±0.63	69.49±1.94	75.66±1.21
PM		79.13±1.80			
	A	-	70.61±0.72	75.31±2.17	76.20±0.22
	B	-	74.98±2.07	77.66±1.95	76.25±1.79
	C	-	74.66±0.57	78.67±0.70	75.40±1.42
PM – U		77.05±1.45			
	A	-	75.76±1.66	75.60±1.12	76.08±1.60
	B	-	72.28±2.14	74.46±1.19	75.83±1.57
	C	-	75.87±1.77	78.17±1.13	76.52±0.60

[1]SM = soybean meal; SMw = whole soybean meal; PM = peanut meal. A, B and C = mixtures of tannin with different ratios of condensed tannin/hydrolysable tannin (CT/HT), respectively 85/15; 55/45; 20/80.

Table 4. Digestible rumen undegradable protein (RUPd, %) of three protein meals, moistened (U) or not, treated with tannin mixtures (A, B and C) and inclusion levels for each meal (1, 2.5 and 5%).

Item		Tannin level			
		0%	1%	2.5%	5%
SM		19.15±0.95			
	A	-	22.67±0.93	21.55±0.48	21.04±1.29
	B	-	19.62±0.81	21.68±0.20	24.29±0.69
	C	-	20.83±0.24	21.40±0.09	23.62±1.74
SM - U		18.64±1.56			
	A	-	48.02±1.58	58.05±3.15	52.62±5.90
	B	-	49.77±4.30	47.61±1.10	52.84±1.15
	C	-	45.13±0.53	44.31±1.05	52.27±1.65
SMw		34.70±1.07			
	A	-	35.17±1.06	36.13±0.35	37.82±1.55
	B	-	35.45±1.01	35.49±1.12	36.59±1.70
	C	-	35.00±0.37	38.88±1.01	37.83±0.68
SMw - U		32.39±4.03			
	A	-	37.58±0.09	38.31±9.12	44.69±0.11
	B	-	38.83±0.64	38.99±0.61	44.67±0.14
	C	-	39.13±0.69	38.44±1.42	43.44±2.69
PM		22.83±2.34			
	A	-	25.04±0.29	33.00±2.10	37.81±3.15
	B	-	29.98±1.05	32.94±0.27	37.66±0.28
	C	-	34.41±0.43	35.91±0.48	37.59±1.31
PM - U		35.26±1.01			
	A	-	46.95±0.33	47.05±0.26	44.63±0.56
	B	-	40.29±1.20	45.32±0.76	52.23±1.62
	C	-	40.48±1.31	45.70±0.09	47.90±1.80

[1]SM = soybean meal; SMw = whole soybean meal; PM = peanut meal. A, B and C = mixtures of tannin with different ratios of condensed tannin/hydrolysable tannin (CT/HT), respectively 85/15; 55/45; 20/80.

Table 5 shows treatment clusters based on RDP and RUPd. Eight groups were formed. Group I and Group VIII were formed respectively by treatments with the most desirable characteristics and by treatments with the least desirable characteristics. Group I was made up of two treatments; Group II by five treatments; Group III by four treatments; Group IV by one treatment; Group III by four treatments; Group IV by one treatment; Group V by 31 treatments; Group VI by four treatments; Group VII by 11 treatments; Group VIII by one treatment.

The variable with the highest rate for discrimination was DPR (80.2%), followed by NDPRd (19.8%).

Groups I, II, III and IV were formed only by samples with solubilization treatment in a water medium and treated with one type of tannin. However, the last three groups (VI, VII and VIII) were formed only by treatments without solubilization treatment of the samples. However, tannin was added to some of these treatments.

Tannin is basically formed by polyphenolic nucleic with several a-polar zones, such as benzene annulene, which may react with the proteins´ a-polar zones (Archana et al., 2010). Protein and polyphenol a-polar regions in the presence of water associate themselves through Van der Walls´s bonds and decrease the surface of the a-polar zones exposed to water. Water molecules associated with the a-polar zones in an orderly way were released to join the solvent, and thus originate the hydrophobic effect.

Hydrophobic interactions between tannins and proteins are the main factors that complexate the two structures. Since hydrophobic interactions occur only in the presence of water, treatments with tannin and moist are justified. Treatments which underwent solubilization in water had the best clusters since the best interactions between tannin and protein occurred when water was present. Complexation decreased protein degradation by rumen bacteria and increased non-degraded protein in the rumen.

On the other hand, when the mixtures of the treatments without water are ingested by the animal and reach the rumen lumen, they mix with the water medium that forms the meal cake and, at this instance, they complex with the proteins for the above-mentioned effects. Since there are other proteins in the rumen, free tannin loses effectiveness in complexing the proteins of the meal under analysis. It may be very relevant to treat meals with water prior to giving them to the animals. It actually capacitates tannin complexation with the meal´s proteins.

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Table 5. Treatment clustering based on RDP, intestinal digestibility of the RUP in the rumen and digestible RUP (RUPd).

Groups	GI	GII	GIII	GIV	GV	GVI	GVII	GVIII	Contribution of the variables (%)
RDP (% of CP)	20.27	29.29	21.46	28.94	43.44	57.04	70.34	72.63	80.2
RUP (% of CP)	79.73	70.71	78.54	71.06	56.56	42.96	29.66	27.37	-
RUP digestibility	79.96	76.06	64.85	56.13	75.59	75.90	73.24	59.09	-
RUPd (% of CP)	63.75	53.78	50.93	39.89	42.75	32.61	21.72	16.17	19.8
Treatments belonging to the groups	SM-U A 2.5%	PM-U A 5%	SM-U B 2.5%	SM-U C 1%	SMw-U A 1%	SMw-U B 1%	PM B 2.5%	SM 0%	SM C 2.5%
	SM-U A 5%	PM-U B 5%	SM-U B 5%		SMw-U C 5%	PM-U C 2.5%	PM C 2.5%	SM A 1%	
		PM-U C 5%	SM-U C 2.5%		SMw-U C 2.5%	SMw-U B 2.5%	PM A 2.5%	SM A 2.5%	
		SM-U A 1%	SM-U C 5%		PM-U C 1%	PM-U B 1%	PM B 1%	SM A 5%	
		SM-U B 1%			PM-U B 2.5%	SMw-U B 5%		SM B 1%	
					SMw-U C 1%	SM-U 0%		SM B 2.5%	
					SMw-U A 2.5%	PM B 5%		SM C 5%	
					SMw-U A 5%	SMw B 5%		SM B 5%	
					PM-U A 1%	SMw C 1%		SM C 1%	
					PM-U A 2.5%	SMw C 2.5%		PM 0%	
					SMw C 5%	SMw B 1%		PM A 1%	
					PM-U 0%	PM C 5%			
					SMw B 2.5%	SMw 0%			
					PM A 5%	SMw A 1%			
					PM C 1%	SMw A 2.5%			
					SMw A 5%				

The addition of water, even without tannin, caused a slight decrease in RDP and consequently an increase in RUP (Table 2). The above was probably due to the drying of samples which raised the sample´s temperature and may have triggered Maillard´s reaction which complexes amino acids and proteins with carbohydrates and thus decreases its availability. Increase in the amount of non-digestible RUP (RUPnd) shown in Figure 2 may have been caused by this effect.

When soybean meal and its respective treatments (Figure 1a) were evaluated, non-solubilized samples remained in the last two clusters (Table 5). On the other hand, samples of solubilized soybean meal which received at least 1% of tannin mixture were placed in the first four clusters. In fact, treatments SM-U A 2.5% and SM-U A 5% had the best characteristics of RUP (group I, Table 5) with average 20.27% of RDP (Table 5), whereas soybean meal without any treatment had 72.87% of RDP when compared to total CP (Table 2). In other words, there was a 52.6% decrease in RDP rates and therefore an increase in the amount of RUP and digestible RUP (Figure 2).

When compared to peanut meal (Tables 2 and 5), the greatest effect of tannin complexation in soybean meal was probably due to the amino acids in the meals. In fact, tannins have a great complexation capacity in the presence of specific amino acids within the protein´s lateral chain and which favored approximation of the a-polar zones for the occurrence of the interactions. Soybean meal (SM) has approximately 14.4% of DM of amino acids with a-polar side chains (glycine, alanine, valine, leucine, isoleucine and proline), whereas peanut meal has only 9.87 % (Valadares Filho, 2006). Therefore, greater quantities of these amino acids in SM may have enhanced a great number of hydrophobic interactions which caused a greater amount of RUP of meal when treated with tannin.

Further, soybean meal has a greater proline concentration when compared with peanut meal (2.4 *versus* 1.4% of DM; Valadares Filho, 2006). Several authors verified hydrophobic interactions, especially in proteins with greater rates of proline (Baxter, Lilley, Haslam & Williamson, 1997; Charlton et al., 2002; Hatano & Hemingway, 1996; Jöbstl, Howse, Fairclough & Williamson, 2006; Murray, Williamson, Lilley & Haslam, 1994; Oh, Hoff, Armstrong & Haff, 1980; Wróblewski, Muhandiram, Chakrabartty & Bennick, 2001).

The amount of digestible RUP, which represents

the amount of RUP digested in the post-rumen phase, increased from 19.82 to 63.82%, when soybean meal was treated with either 2.5 or 5% of Type A tannin in a moistened medium (Figure 2). The above result shows that treatments were the best options to protect SM protein from degradation by the rumen.

Results on peanut meal revealed that treatment with 5% of any type of tannin (A, B or C) in a water medium caused an efficient complexation of its proteins since they were grouped in G II (Table 5). The highest RUPd rates in soybean meal were reported in treatments with 2.5 and 5% of tannin, whereas satisfactory results in peanut meal were found only at 5% inclusion.

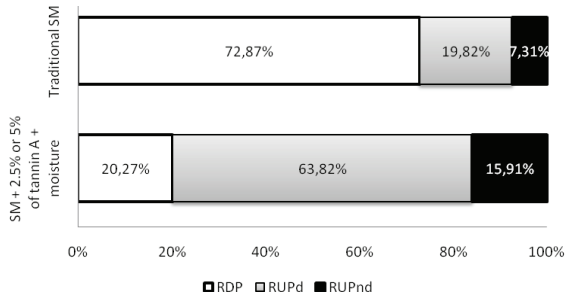

Figure 2. Descriptive evaluation of crude protein of soy meal treated with tannin and moisture (RDP = Rumen degradable protein; RUPd = digestible rumen undegradable protein; RUPnd = non digestible rumen undegradable protein).

Tannins in whole soybean meal (SMw) were not efficient since all treatments with this ingredient are in the same cluster (GV; Table 5). Among the different tannin mixtures, the treatments with the best results were those treated with mixture "A" (85% of condensed tannin and 15% of hydrolysable tannin). Although they have a smaller capacity of bonding with protein than hydrolysable tannins (Khanbabaee & Van Ree, 2001), the condensed tannins have a greater bonding stability when exposed to media with high enzyme activity. Since it may be presupposed that meal treatments with water prior to their exposure in the rumen environment (*in situ* incubation) was effective in the complexation of tannins with protein, it may be that condensed tannins had a greater capacity of maintaining bonds with proteins since the latter were not degraded by rumen microorganisms, different from hydrolysable tannins (Khanbabaee & Van Ree, 2001).

Conclusion

Soybean meal, in a water medium, treated with 2.5% tannin with 85% condensed tannin and 15% hydrolysable tannin increases digestible RUP of diet. The inclusion of the ingredient in *in vivo* evaluation should be tested to determine increase in productivity and the inclusion level adopted.

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