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Effects of different sources of protein on digestive characteristics, microbial efficiency, and nutrient flow in dairy goats

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ABSTRACT - Diets formulated with protein sources presenting different resistance to ruminal degradation were compared by evaluating ruminal parameters, production and microbial efficiency and nutrients flow to the omasum in goats. Eight rumen cannulated non-lactating, non-pregnant goats were distributed in a 4 × 4 Latin square design with two replicates. Treatments consisted of four diets where different sources of plant protein accounted for the major protein source named soybean meal, source of higher ruminal degradability, and three other sources of higher resistance of degradation: roasted soybean, corn gluten meal, and cottonseed cake. Amounts of rumen protein were similar among rations; however, flows of dry matter, protein and non-fiber carbohydrate to omasum were higher for diets with protein source with reduced rumen degradation rate. Higher values of rumen ammonia were obtained by using ration with soybean meal as major source of protein. Higher values of pH were obtained for rations with roasted soybean e cottonseed cake. Regarding kinetic of transit, similar values were found among rations. Diets with protein sources presenting reduced ruminal degradation increase nutrients flow to the omasum in goats and alter digestive parameters such as pH and ammonia without compromising bacteria growth and efficiency, which grants their use for dairy goats with similar efficiency to rations using more degradable sources of protein.

Key Words: ammonia nitrogen, biological value, goats, microbial protein, passage rate, pH

Introduction

In ruminants, the need for amino acids in tissues increases according to their milk production level. NRC (1985) recommends the inclusion of slowly degradable sources in the rumen to increase the flow of nutrients in the small intestine; however, the microbial production of protein should not be affected by the use of these sources.

Dietary protein can undergo different degrees of degradation in the rumen. Thus, there may be interesting cases of intervention during degradation, so that when these proteins are high-quality, they reach the abomasum and intestine to be digested before any transformation can occur. The dietary characteristics do not indicate the quantity and quality of the protein reaching the small intestine for absorption. According to NRC (1996), much of the protein reaching the small intestine is from microbes and food that has not been degraded in the rumen.

Soto-Navarro et al. (2006) reported that the amount of protein of microbial origin cannot meet the needs of ruminant animals under conditions of high protein demand, as in the case of growing animals and those producing milk.

In a review, Santos et al. (1998) found many studies on the effect of using different sources of rumen undegradable protein for milk production. However, literature studies are scarce regarding the effects of these sources on rumen kinetics and parameters and the efficiency and production of microbial protein.

This paper aims to test the effect of different sources of protein on the nutrient flow to the omasum; microbial production and efficiency, and the rumen characteristics, such as pH and ammoniacal nitrogen, in dairy goats.

Material and Methods

The experiment was conducted in the Setor de Caprinocultura of the Departamento de Zootecnia at Universidade Federal de Viçosa, located in Viçosa, Minas Gerais, Brazil, from March 18 to June 12, 2006.

Four isonitrogenous diets (18% crude protein - CP) composed of different protein sources were evaluated (Table 1). The main sources of protein in each diet were the following: soybean meal (*Glycine max* L.), soybean roasted grains, corn gluten meal (gluten 60% CP), and cottonseed

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cake (*Gossypium hirsuntum*). Eight goats of the Saanen and Alpine breeds were used, with an average weight of 51 kg, not pregnant or lactating; the experimental design was a distribution in a balanced 4 × 4 Latin square design, with two replicates.

Goats were vaccinated against tetanus and subsequently cannulated in the rumen with 2.5-inch cannulas. After a period of 7 to 10 days, cannulas were replaced with others of 3 inches for the manual manipulation of rumen contents. Before the beginning of the experiment, animals were identified and treated against endo- and ecto-parasites. During the experimental period, animals were confined to individual stalls (2 m²) with slatted wood floors that were fitted with individual feeders and water dispensers and modified for the collection of total feces and urine.

The experimental diets had a composition similar to an experiment conducted simultaneously with animals in milk production at EMBRAPA Caprinos. Therefore, the nutrient concentrations met the recommendations of the AFRC (1993) for lactating goats. The proportions of crude protein and net energy were held constant (18% CP and 1.80 Mcal/kg, respectively) in all of the diets (Table 2). Fiber content was maintained by offering hay (Tifton 85, *Cynodon* spp.) exclusively, which accounted for 35% of the NDF of the forage. Ground corn (*Zea mays*, L.) was used as the main energy source available in the diets and was supplemented with minerals.

Each experimental period lasted 21 days (11 days for the animals to adapt to the rations and 10 days of sample collection). Animals were fed twice a day at 7:30 a.m. and

Table 1 - Proportions of ingredients in the experimental diets

Ingredients	Main sources of protein in the diet (% DM)			
	Soybean meal	Toasted soybean	Corn gluten meal	Cottonseed cake
Hay Tifton 85	44.67	44.67	44.67	44.67
Corn meal	38.74	36.45	43.45	29.47
Soybean meal	14.90	4.03	-	8.82
Toasted soybean	-	13.12	-	-
Corn gluten meal	-	-	10.19	-
Cottonseed cake	-	-	-	15.35
Macromineral mixture[1]	1.69	1.73	1.69	1.69

[1] Macromineral mixture- 66% dicalcium phosphate, 44% limestone; micromineral mixture- 0.32% ferrous sulfate, 0.48% copper sulfate, 0.71% manganese sulfate, 2.67% zinc sulfate, 0.02% cobalt sulfate, 0.0125% potassium iodate, 0.006% sodium selenite, 95.78% sodium chloride.

Table 2 - Bromatological composition of the experimental diets

Item	The main source of protein in the diet (% DM)			
	Soybean meal	Toasted soybean	Corn gluten meal	Cottonseed cake
	Composition			
Dry matter (%)	87.43	88.00	87.74	87.94
Organic matter (%)	94.26	94.18	94.93	94.28
Crude protein (%)	18.57	18.36	18.19	17.14
Ether extract (%)	3.23	5.60	3.62	3.82
Total carbohydrates (%)	72.47	70.23	73.13	73.33
Non-fibrous carbohydrates (%)	31.77	28.76	32.71	26.01
Neutral detergent fiber (%)	44.83	46.99	45.27	52.00
NDFcp (%)	40.71	41.47	40.42	47.32
NDFf (%)	34.37	34.37	34.37	34.37
ADF (%)	23.34	24.43	22.73	29.66
iADF (%)	5.67	5.68	5.69	8.31
NDIP (%PB)	23.85	26.90	25.45	24.42
ADIP (%PB)	3.54	3.80	3.92	5.15
ADL (%)	2.71	2.75	2.83	4.02
Ash (%)	4.04	4.08	3.37	4.02
Calcium (%)	0.73	0.74	0.70	0.73
Phosphorus (%)	0.53	0.55	0.51	0.52
	Estimated energy			
TDN (%)	71.39	73.89	72.29	67.97
FME (Mcal/kg)	2.56	2.47	2.56	2.35
NE (Mcal/kg)[3]	1.79	1.89	1.82	1.68
FME:CP	0.138	0.135	0.141	0.137

NDFcp = neutral detergent fiber corrected for ash and protein, NDFf = NDF of forage, ADF = acid detergent fiber, iADF = indigestible ADF, NDIP = neutral detergent insoluble protein, ADIP = acid detergent insoluble protein, ADL = acid detergent lignin, TDN = total digestible nutrients, FME = fermentable metabolizable energy, NE = net energy.

3:30 p.m., and received a complete mixture of hay (Tifton 85) and concentrate mixture in sufficient quantity so that there were 10% of leftovers to ensure *ad libitum* food intake by the animals.

Flow of the nutrients to the omasum, microbial production and efficiency, pH and ammoniacal nitrogen were observed, while evaluating and comparing the effects of protein sources. Samples of the feeds, leftovers, digesta, and feces were dried at 60 °C in a convection oven for 72 hours, processed in a Willey mill with sieves of a mesh size of 1 mm and individually packed into glass bottles at room temperature.

The laboratory analyses were performed at the Laboratório de Nutrição Animal, Departamento de Zootecnia at UFV to determine the chemical composition of the dry matter (DM), total nitrogen (TN), ether extract (EE) and ash using the techniques described by Silva & Queiroz (2002). Neutral detergent fiber (NDF) and acid detergent fiber (ADF) were determined according to Van Soest et al. (1991), the neutral detergent insoluble nitrogen (NDIN) and acid detergent insoluble nitrogen (ADIN) were determined according to techniques described by Licitra et al. (1996), and the acid detergent lignin was determined according to the methodology described by Van Soest (1965). Feces, rumen, and omasum digesta were analyzed for DM, CP, EE, ash, and NDF. The estimates for total carbohydrates (TC) and non-fiber carbohydrates (NFC) were obtained with the equations proposed by Sniffen et al. (1992) and Van Soest et al. (1991), respectively:

$$TC = 100 - (\%CP + \%EE + \%Ash)$$

$$NFC = 100 - (\%CP + \%EE + \%Ash + NDF\ cp)$$

The energy values of the ingredients of the diet were estimated by the equations proposed by the NRC (2001), considering the class of food descriptions as forage, concentrate, animal products, or fatty acid.

Flow of nutrients to the omasum and rate of passage of solids were determined using the indigestible acid detergent fiber (iADF) as an indicator, a technique described by Cochran et al. (1986). However, rumen incubation in Ankon®-type bags, with dimensions of 5 × 5 cm, in TNT-100 (non-woven fabric) was performed for 144 hours, instead of using the *in vitro* digestibility suggested in the original protocol. For the quantification of omasum flow, aliquots of approximately 150 mL of the omasum digesta were obtained simultaneously with the collection of feces samples using a set of devices consisting of a kitasato, a header tube and a vacuum pump, according to procedures described by Silva et al. (2007).

Immediately after collection, samples were stored in plastic containers and later used as composite samples per period and per animal based on pre-dried matter.

Dry matter flow was calculated according to the following equation:

$Flow = \frac{DII \times 100}{[IND_{om}]}$, where DII is the daily intake of the indicator and $[IND_{om}]$ is the concentration of the indicator in the omasum digesta.

The passage rate (kp) was estimated by the rumen evacuation technique, according to the methodology described by Robinson et al. (1987). To reduce the effects of food, we made three collections of the rumen contents, the first at 4:30 a.m., the second at 1:30 p.m., and the third at 11:30 p.m. on the 7th, 8th, and 9th day of the collection period, respectively.

Once removed, the rumen contents were separated into solid and liquid fractions, which were individually weighed and sampled (400 g) proportionally. Samples were incubated in a convection oven (60 °C) and then processed through a Willey mill with mesh sieves of 1 mm. Samples were organized for laboratory analysis on a dry basis, equally per goat for each period. The mass of the rumen nutrients was calculated based on the results of the analysis.

Passage rate (kp) was estimated by the ratio of daily omasum flow of the indicator divided by 24 hours and by the rumen mass indicator, according to the requirements suggested by Cannas & Van Soest (2000):

$$k_p = \frac{\dfrac{\text{Daily flow of indicator (g.d}^{-1}\text{)}}{24\,hours}}{\text{Rumen mass indicator (g)}}$$

Concentration of ammoniacal nitrogen (NH3-N) and the rumen pH were measured at 2-hour intervals after the morning feeding over 24 hours. pH was determined directly in the rumen of the animal using a digital portable potentiometer (PH-1400; Instru Therm). For the analysis of the levels of ammonia, rumen fluid samples were collected at the same time as pH measurement, filtered through four gauze layers, and acidified with 50% sulfuric acid at a ratio of 0.4 mL of acid to 20 mL of fluid. Samples were frozen and analyzed using the technique described by Chaney & Marbach (1962).

On the sixth day of the collection period, 750 mL of the rumen digesta fluid were removed before feeding, and another 750 mL were collected 6 hours after feeding for the isolation of the rumen bacteria, as described by Cecava et al. (1990). The estimates of bacterial nitrogen production were done according to the technique of the purines (Zinn & Owens, 1986) in the bacterial pellet and omasum digesta.

The efficiency of the microbial synthesis was calculated from the carbohydrate and organic matter degraded in the rumen and estimated by the difference between the consumption of carbohydrates or organic matter and the omasum flow of these fractions.

The results were evaluated by analysis of variance using the Statistical Analysis System, SAEG 9.1 (UFV, 2007), and by applying the Student-Newman-Keuls test (SNK) at 5% probability to compare the means.

Data regarding pH and concentrations of ammonia in the rumen fluid were analyzed in a split-plot design, in which the treatments corresponded to plots and the timing, to subplots. For treatment factor, the means were compared using the Student-Newman-Keuls test (SNK) at 5% probability, and a regression analysis was adopted for the time factor. Models were chosen based on the significance of the regression coefficient using the t-test at 5% probability on the coefficient of determination and biological phenomenon.

Results and Discussion

The flow of the DM, OM, NDF, EE, NFC, and total carbohydrates in the omasum of goats fed on different protein sources (in g/d) showed differences ($P<0.05$) between the diets (Table 3). Greater flows of the DM and OM (g/d) were observed ($P<0.05$) when using the diets with roasted soybeans and cottonseed cake. When the diet containing corn gluten meal was provided, intermediate values were obtained; however, these values were similar to those obtained with the diet containing soybean meal. Nevertheless, this diet showed a lower DM and OM flow ($g.d^{-1}$) when compared with the diet containing roasted soybeans.

NDF flow into the omasum was greater with the diets composed of cottonseed cake and roasted soybeans, compared with the diet with soybean meal, whereas the results with corn gluten meal did not differ from any of the other diets. This result may be related to the reduced flow of crude protein, which, although not statistically different ($P>0.05$), showed a tendency to decrease in a manner similar to the NDF. The timing of the utilization of protein and energy can provide good fiber degradation by fibrolite bacteria, and the concomitant use of degradable protein in the rumen, such as soybean meal, promotes the reduced flow of nutrients into the omasum. Accordingly, it was also observed that the NFC flow (g/d) from the diet containing soybean meal was lower than the diets with undegradable sources, which did not differ between themselves.

The greatest value for the flow of ether extract was obtained with the diet containing the roasted soybeans, probably because of the high concentration of this nutrient in this protein source. The ether extract is not used as an energy source by microorganisms, so the amount of this nutrient in the omasum is very close to that of the diet in the form that it was consumed (Table 4).

The flows of the DM, OM, CP, NFC, and TC that were relative to the amount consumed showed differences ($P<0.05$) between the diets. For all of the variables with significant differences, there was reduced nutrient flow based on the materials consumed in the diet of soybean meal, which has higher protein degradability, whereas similar values for the nutrient flow were observed for the diets with the lowest rumen degradability.

The amount of nitrogen in the RNA (g/kg), the total nitrogen in the bacterial pellet (g/kg), the ratio of the nitrogen RNA to total nitrogen, the microbial nitrogen flow, and the efficiency of microbial production (the g/kg OMDR, g/kg TCDR, and g/kg TDN) did not differ ($P>0.05$) between the diets (Table 5). The flow of microbial nitrogen, with values ranging from 7.37 to 10.13 $g.day^{-1}$, confirms the data obtained by Fonseca et al. (2006) of 8.6 to 12.75 g/day in a survey evaluating diets containing 12-18% crude protein. Rodrigues et al. (2007), working with a different CP/NE ratio, observed flows of microbial nitrogen of 10.24 and 8.24 g/day in diets containing 16.5 and 19.5% crude protein, respectively.

Averaging 117 g/kg NDT, the data in the present study were very close to the estimates of the microbial efficiency by the NRC (2001) for the same characteristics, with a value

Table 3 - Flow of dry matter and nutrients in the omasum of dairy goats fed diets with different protein sources

Item	The main source of protein in the diet				CV (%)	Pr ≥ Fcal
	Soybean meal	Toasted soybean	Corn gluten meal	Cottonseed cake		
Dry matter (g/d)	214.90b	361.00a	289.60ab	373.40a	28.13	0.01
Organic matter (g/d)	167.70b	293.00a	234.30ab	297.10a	28.26	0.01
Neutral detergent fiber (g/d)	86.60c	140.30ab	109.60bc	160.50a	27.59	0.01
Crude protein (g/d)	52.16	84.81	75.96	80.21	32.11	ns
Ether extract (g/d)	14.20c	31.30a	17.80bc	22.60b	29.06	0.001
Fibrous carbohydrates (g/d)	18.00b	36.60a	30.90a	33.80a	36.52	0.02
Total carbohydrates (g/d)	101.30b	176.90a	140.50ab	194.30a	28.76	0.005

Means followed by the same letter in the row do not differ at 5% significance by SNK test. CV = coefficient of variation; ns = not significant.

of 130 g microbial protein per kg of TDN, which indicated no limitation in microbial production.

The amount of dry matter and nutrients in the rumen obtained with different protein sources was similar (P>0.05) (Table 6). The average percentage of dry matter present in the rumen was 11.27%, and the average rumen mass represented 8.44% of the weight of the animals.

The rumen fluid differed (P <0.05) between the protein sources evaluated. The amount of liquid in the rumen differed between the animals fed on soybean meal and cottonseed cake as the main source of protein, in which the cottonseed cake diet resulted in the lowest amount of liquid. These two sources did not differ from the other protein sources evaluated (roasted soybeans and corn gluten meal). It is likely that the reduced flow of dry matter observed in the animals fed on soybean meal may explain the largest accumulation of fluid in the rumen. However, these results do not agree with what has been proposed by Church (1993), that the consumption of diets with higher amounts of fiber (in this case, the diet containing cottonseed cake) results in a higher amount of liquid in the rumen due to increased salivation.

Table 4 - Flow of the dry matter and nutrients to the omasum as a percentage of dry matter intake (DMI%)

Item	The main source of protein in the diet				CV (%)	Pr ≥ Fcal
	Soybean meal	Toasted soybean	Corn gluten meal	Cottonseed cake		
Dry matter (%DMI)	37.28b	54.71a	49.49ab	56.74a	22.95	0.02
Organic matter (%DMI)	30.94b	47.14a	42.07a	47.83a	22.99	0.01
Neutral detergent fiber (%DMI)	36.90	51.62	47.70	51.59	23.72	ns
Crude protein (%DMI)	48.53b	70.07a	67.35a	70.55a	22.58	0.03
Ether extract (%DMI)	77.42	85.28	82.27	93.47	30.25	ns
Non-fibrous carbohydrates (%DMI)	10.54b	21.19a	16.60a	19.31a	30.40	0.01
Total carbohydrates (%DMI)	24.36b	38.13a	33.79a	40.14a	25.32	0.01

Means followed by the same letter in the row do not differ at 5% significance by SNK test. CV = coefficient of variation; ns = not significant.

Table 5 - Production, flow, and efficiency of microbial protein production based on omasum dry matter flow in dairy goats fed on diets with different protein sources

Item	Main source of protein in the diet				CV (%)	Pr ≥ Fcal
	Soybean meal	Toasted soybean	Corn gluten meal	Cottonseed cake		
NRNA Bact (%)	12.80	12.20	11.70	14.60	21.53	ns
Ntotal Bact (%)	86.80	85.00	83.90	85.10	9.09	ns
NRNA/NTotal	0.15	0.15	0.14	0.17	20.97	ns
N mic Flow ($g.d^{-1}$)	7.26	10.13	9.46	7.37	56.64	ns
Pmic/OMDR (g/kg)	121.11	134.66	172.26	143.41	50.85	ns
Pmic/TCDR (g/kg)	195.78	267.32	231.73	267.66	48.47	ns
Pmic/TDN (g/kg)	118.24	133.48	126.77	87.80	44.21	ns

Means followed the same letter in the row do not differ at 5% significance by SNK test. CV = coefficient of variation; NRNA Bact = RNA bacterial nitrogen; Ntotal Bact = total bacterial nitrogen; NRNA/NTotal = RNA nitrogen/ total nitrogen ratio; N mic Flow = nitrogen flow; Pmic/ OMDR = microbial protein per kg of organic matter; Pmic/TCDR microbial protein per kg of total carbohydrate degraded in the rumen; Pmic/TDN = microbial protein per kilogram of total digestible nutrients; ns = not significant.

Table 6 - Weight, mass and rumen contents (averages) of dairy goats fed on diets with different protein sources

Item	Main source of protein in the diet				CV (%)	Pr ≥ Fcal
	Soybean meal	Toasted soybean	Corn gluten meal	Cottonseed cake		
Body weight (kg)	46.80	47.93	50.32	48.95	10.41	ns
Solid content (kg)	1.45	1.41	1.45	1.73	20.78	ns
Wet content (kg)	2.77a	2.63ab	2.42ab	2.26b	13.12	0.04
Total (kg)	4.23	4.27	3.86	4.03	13.22	ns
Ruminal mass						
Dry matter (g)	350.47	366.66	339.93	397.16	21.49	ns
Organic matter (g)	301.75	318.28	292.93	351.25	23.09	ns
Crude protein (g)	74.88	71.14	73.13	75.33	26.61	ns
Ether extract (g)	8.25	12.89	10.11	7.31	53.77	ns
Neutral detergente fiber (g)	181.44	196.09	177.81	227.79	21.94	ns
Non-fibrous carbohydrates (g)	37.18	38.16	31.88	40.83	37.24	ns
Total carbohydrates (g)	218.61	234.25	209.69	268.62	21.59	ns

Means followed by the same letter in the row do not differ at 5% significance by SNK test. CV = coefficient of variation; ns = not significant.

The concentration of ammoniacal N showed differences (P<0.05) between the diets (Table 7). The highest values were obtained when the goats were fed on diets with soybean meal compared with less degradable protein sources (Figure 1). Considering that the soybean meal has a higher fraction of rapidly rumen-degradable protein, there was probably a larger pool of ammoniacal nitrogen in this compartment of the digestive tract. In the present study, the values obtained for diets with sources of low degradability were higher than the minimum values of ammoniacal nitrogen recommended for the maintenance of the normal functions of the rumen, 5 mg/dL, according to Satter & Slyter (1974). However, our results are consistent with Leng (1990), who recommended levels of ammonia concentrations that are higher than 10 and 20 mg/dL for tropical regions to maximize the digestion and consumption of dry matter, respectively.

The sources of protein in the diets promoted differences (P<0.05) in the pH (Table 7), as significant results were obtained when giving diets with soybeans and toasted cottonseed cake (averages of 6.23 and 6.18, respectively), whereas the average obtained with all of the diets (6.17) was very close to the minimum pH recommended by Van Soest (1994), 6.7 (which may deviate by 0.5). Low values were observed during the day (Figure 2), with instances of acidification that could compromise the activity and motility of the fibrolytic microbes in the rumen, in addition to suboptimal enzyme activity of a low pH.

There were no differences (P >0.05) between the passage rates obtained with the different protein sources (Table 7). The average rates recorded in this study were similar to those described in the AFRC (1993): $kp = -0.024 + 0.179*(1-e^{-0.278*L})$, where the estimated passage rate was a function of L, a multiple of the total needed energy as a function of maintenance. As the animals were at a maintenance level, i.e., with reduced activity, the value estimated by the equation of AFRC kp = 0.0219/h was very close to that observed in this study, with a mean of 0.025 kp/h.

Table 7 - Rumen parameters of dairy goats fed on diets with different protein sources

Item	Main source of protein in the diet				CV (%)
	Soybean meal	Toasted soybean	Corn gluten meal	Cottonseed cake	
$N\text{-}NH_3$ (mg/dL)	26.68a	21.05b	20.11b	21.21b	34.84
pH	6.12b	6.23a	6.13b	6.18ab	4.63
k_p (h^{-1})	0.024	0.025	0.023	0.029	33.51

Means followed by the same letter in the row do not differ at 5% significance by SNK test. CV = coefficient of variation; N-NH3 = ammoniacal nitrogen; k_p = passage rate.

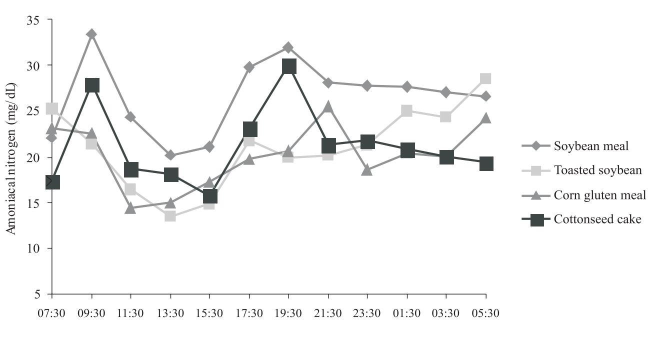

Figure 1 - Effect of diets containing different protein sources on the ammonia concentration (mg/dL) in the rumen.

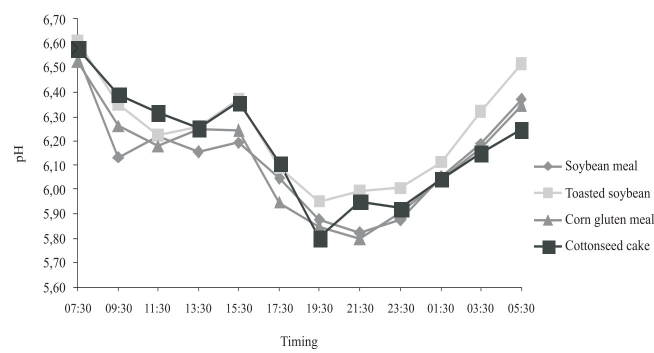

Figure 2 - Effect of diets containing different protein sources on the behavior of rumen pH.

Conclusions

The use of protein sources with lower rumen degradability promotes an increase in the flow of nutrients and changes in the digestive parameters of the omasum, but does not compromise the production or microbial efficiency in dairy goats.

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