

PROTEIN SUPPLEMENTATION FOR SHEEP FED WITH TROPICAL FORAGE

Daniel Marino Guedes Carvalho

Universidade Federal do Mato Grosso - UFMT,
Cuiabá, MT, Brazil,

Janaina Januário da Silva

Universidade Federal do Mato Grosso - UFMT,
Cuiabá, MT, Brazil,

<https://orcid.org/0000-0002-2250-298X>

Email correspondente: janajanu@yahoo.com

Marivaldo Jacaúna Santos

Universidade Federal do Amazonas, Manaus, AM,
Brazil,

<https://orcid.org/0000-0002-4913-1488>

Caio de Souza Teixeira

Universidade Federal do Amazonas, Parintins, AM,
Brazil,

<https://orcid.org/0000-0002-3627-245X>

Evelyn Prestes Brito

Universidade Federal do Amazonas, Parintins, AM,
Brazil,

<https://orcid.org/0000-0002-5728-5735>

Nelma Pinheiro Fragata

Universidade Federal do Amazonas, Parintins, AM,
Brazil,

<https://orcid.org/0000-0001-5729-7085>

Received: 27/06/2019

Approved: 07/07/2020

Abstract

The effects of increasing levels of protein supplementation (50, 100, 150 and 200 animal per day) and mineral salt on feed intake, nutrient digestibility and ingestive behavior of sheep consuming tropical forage (*Pennisetum purpureum* Schum) were evaluated. Five non-castrated crossbred lambs ($\frac{1}{2}$ Santa Inês x $\frac{1}{2}$ undefined breed) with average initial body weight of 35.0 kg (\pm 4.40 kg) were allocated in individual cages and analyzed in a 5x5 Latin square experimental design composed of five animals, five treatments and five evaluation periods of 14 days each. The contrast (protein supplement vs. mineral mixture) was not significant ($p > 0.05$) for total dry matter intake, total organic matter intake, total ether extract intake and total carbohydrate intake variables. However, it was significant ($p < 0.05$) for the variables forage dry matter intake, total crude protein intake, total mineral matter intake, total neutral detergent fiber intake and total non-fibrous carbohydrate intake, demonstrating possible replacement of forage by the supplement. Although the diet dry matter digestibility declined linearly ($p < 0.05$), there was a significant linear increase effect of supplementation ($P < 0.05$) on apparent digestibility of neutral detergent fiber, non-fibrous carbohydrates and consumption of digestible non-fibrous carbohydrates. This was due to higher participation of supplement in the diet ingested by supplemented sheep. The type of supplementation (protein or mineral mixture) did not affect the animals' daily activities. Protein supplementation did not affect the water ingestion ($P < 0.05$), demonstrating that even at the highest level of supplementation (200 g/day), animals were not induced to drink more water by the diet. We conclude that increasing levels of protein supplementation associated with good quality elephant grass resulted in increases in sheep digestive parameters, and supplementation below 100 g day⁻¹ resulted in similar parameters to those of sheep receiving mineral mix (control). Additionally, protein supplementation did not affect ($P > 0.05$) either the water intake or ingestive behavior of sheep, with rumination and rest coinciding with dusk and dawn.

Keywords

digestibility, feed efficiency, intake, rest, rumination

SUPLEMENTAÇÃO PROTEICA PARA OVINOS ALIMENTADOS COM FORRAGEM TROPICAL

Resumo

Foram avaliados os efeitos de níveis crescentes de suplemento proteico (50, 100, 150 e 200 gramas/animal dia⁻¹) e do suplemento testemunha (mistura mineral) sobre o consumo, a digestibilidade dos nutrientes e o comportamento ingestivo de ovinos consumindo forragem tropical (*Pennisetum purpureum* Schum). Foram utilizados cinco borregos meio sangue Santa Inês x Sem Raça Definida, não castrados, com peso corporal inicial médio de 25,33 kg (\pm 4,40 kg), alocados em baias individuais e distribuídos em delineamento experimental quadrado latino 5x5, sendo cinco animais, cinco tratamentos e cinco períodos de avaliação de 14 dias cada. Observou-se que para as variáveis consumo de matéria seca total, consumo de matéria orgânica total, consumo de extrato etéreo total e consumo de carboidratos totais total, o contraste (suplemento proteico x mistura mineral) não foi significativo ($p > 0,05$), contudo, este foi significativo ($p < 0,05$) para as variáveis consumo de matéria seca de forragem, consumo de proteína bruta total, consumo de matéria mineral total, consumo de fibra em detergente neutro total e consumo de carboidratos não fibrosos total, demonstrando uma possível substituição da forrageira pelo suplemento. Apesar da digestibilidade da matéria seca da dieta ter sofrido redução linear ($p < 0,05$), houve efeito significativo de caráter crescente e linear da suplementação ($P < 0,05$) sobre a digestibilidade aparente da fibra em detergente neutro, digestibilidade aparente dos carboidratos não fibrosos e consumo de carboidratos não fibrosos digestíveis. Isto decorreu da maior participação do suplemento na dieta total ingerida pelos ovinos suplementados. O tipo de suplementação (proteica ou mistura mineral) não afetou a realização das atividades diárias dos ovinos e não houve variação no tempo gasto com consumo de água entre os tratamentos ($P > 0,05$), portanto, mesmo no nível mais elevado de fornecimento de suplemento (200 g/dia) os animais não consumiram mais água pelo maior aporte proteico do mesmo. Concluiu-se que os níveis crescentes de suplementação proteica associados ao capim Elefante (*Pennisetum purpureum* Schum) de boa qualidade promovem incrementos nos parâmetros digestivos dos ovinos, contudo, a oferta de suplemento abaixo de 100 g/dia resulta em parâmetros semelhantes aos da mistura mineral. Adicionalmente, a suplementação proteica não afeta ($P > 0,05$) a ingestão de água dos ovinos e o seu comportamento ingestivo, com ruminação e ócio coincidindo com o anoitecer e o amanhecer.

Palavras-chave

consumo, digestibilidade, eficiência alimentar, ócio, ruminação

INTRODUCTION

Although sheep herd were one of the first exotic animal species introduced in Brazil by the Portuguese colonizers, until the 1990s the consumption of lamb and mutton was insignificant in the country, mainly restricted to rural dwellers or during festive occasions such as Easter and Christmas (Bánkuti et al., 2013). Currently, consumption of goat and sheep meat is expanding by more than 10% a year, thus making the market increasingly attractive to small ruminant breeders (SEBRAE, 2018).

However, the great majority of production systems in Brazil fall short of offering products with the quality and uniformity demanded by this new consumer market. There are many small flocks formed by animals without defined breed, or crosses of non-specialized breeds, with low weight gain, prompting slaughter at advanced age and producing carcasses with low yield and meat with poor appeal (IBGE, 2018).

A predominant characteristic of these systems is extensive grazing, in which the animals are left loose where they are dependent on the seasonal variations of native or cultivated pastures. In this situation, the absence of feed supplementation during periods of scarcity or low nutritional quality of forage causes failure to satisfy the nutritional demands, increasing the time for slaughter (LEITE and MEDEIROS, 2014).

The adoption of supplementation depends on the type of supplement employed, the level of nutrients, its composition and the gains provided. Each of these factors should be considered when choosing production systems that rely on supplementation, to attain greater efficiency and maximize farmers' income (CARVALHO et al., 2015).

In this context, protein deficiency of feeds and supplements can hamper the animal productive performance. On the other hand, the excessive supply of protein, besides increasing costs, can impair the reproductive performance, increase the animals' energy demand and cause excessive excretion of nitrogen in the environment (VOLTOLINI et al., 2010). Thus, it is necessary to adjust the protein content of feeds and supplements for ruminants, especially those based on forage plants.

The objective of this study was to evaluate the effects of rising levels of protein supplementation on the consumption, nutrient digestibility and ingestive behavior of sheep fed with tropical forage.

MATERIAL AND METHODS

The study was conducted from December 2011 to February 2012 at the Experimental Ovinoculture Sector of the Undergraduate Animal Husbandry Program of the Institute of Social Sciences, Education and Zootchnics (ICSEZ) of Federal University of

Amazonas (UFAM), located in Parintins, Amazonas State, at 02° 37' 42" South Latitude and 56° 44' 09" West Longitude and altitude of 27 meters. The climate in the region is classified as equatorial by the Köppen system, with average yearly temperature of 26 °C, average annual rainfall of 2,327 mm and relative humidity of 80%.

The study was approved by the Animal Experimentation Ethics Committee of Federal University of Amazonas (CEEAA - UFAM), by protocol no. 084/2012.

The animals were kept in five individual stalls (metabolic cages), each with floor space of 1.5 m², equipped with individual water troughs and two feed troughs, one for supply of fodder (with capacity of 8.0 kg, positioned facing the front of each stall) and the other holding supplement (with capacity of 2.0 kg, placed behind the stall). These stalls were allocated in a brick shed with floor space of approximately 30.0 m², covered with cement fiber cement roof tiles. Five crossbred male lambs were used (Santa Inês x undefined breed) with approximate ages of six months, uncastrated, with initial mean body weight of 25.33 kg (\pm 4.40 kg). Before the start of the experiment, all the animals were treated for ectoparasites using pyrethroids and using 1.0% moxidectin for ectoparasites with the dosage and administration route determined according to the manufacturer's recommendation.

The experimental period was divided into five periods of 14 days, with the first seven days of each period devoted to adaptation of the animals to the supplementation (CARVALHO et al., 2019) and the remaining days reserved for collection of samples. The treatments consisted of rising levels of protein supplement, corresponding to 50, 100, 150 and 200 grams/animal day⁻¹, besides control supplement composed of a mineral mixture. The supplements were supplied daily at 10:00 a.m., and the leftovers were monitored to determine the daily consumption by each animal, while the mineral mixture was supplied *ad libitum*. The protein supplement was composed of soy meal, cornmeal, urea and ammonium sulfate (9:1) and mineral mixture (Table 1), produced in a vertical feed mixer with plastic collection box and capacity to produce 1000 kg of feed per batch.

Table 1. Percentage composition of the supplement made from natural material

Ingredients	Quantity (kg)
Soy meal	30.00
Cornmeal	55.00
Urea+ ammonium sulfate (9:1)	5.00
Mineral mixture ¹	10.00
Total	100.00

¹Commercial mineral mixture for sheep (guaranteed levels per kg of product: 155 g of calcium; 65 g of phosphorus; 115 g of sodium; 6 g of magnesium; 175 mg of cobalt; 100 mg of copper; 175 mg of iodine, 1400 mg of manganese; 42 mg of nickel; 27 mg of selenium; 6000 mg of zinc; fluoride (max) of 650 mg.

The supplement ingredients were weighed and placed in the feed mixer for 15 minutes for homogenization. Then the experimental supplement mixtures were stored in 40 kg bags, each identified according to the corresponding treatment. The bromatological composition of the ingredients and the supplement are reported in Table 2.

Table 2. Bromatological composition of the ingredients and supplement used in the experiment

Items	Ingredients		Supplement
	Cornmeal	Soy meal	
Dry matter (% of NM)	85.05	88.13	85.62
Organic matter ¹	98.83	93.72	86.94
Crude protein ¹	12.50	49.85	30.21
Neutral detergent fiber ¹	13.81	33.06	14.86
Indigestible neutral detergent fiber ¹	1.10	3.85	7.22
Ether extract ¹	4.24	2.45	2.81
Non-fibrous carbohydrates ¹	71.62	31.68	42.88
Total carbohydrates ¹	82.11	41.43	54.42
Mineral matter ¹	1.15	6.28	12.56

¹% expressed in % of dry matter; NM: Natural matter.

The fodder used as voluminous feed consisted of chopped elephant grass (*Pennisetum purpureum* Schum) with particle size of 2 to 3 cm, without further processing (*in natura*). The bromatological composition was identified in Table 3. The elephant grass was cut, and before being chopped to supply the animals, the culms were removed so that the chopped part supplied to the animals was only composed of the blade tips. The resulting fodder was supplied to the animals in the metabolic cages twice a day, at 7:00 a.m. and 5:00 p.m., in volume sufficient to provide daily leftovers of 10%, which were weighed before replenishing the fodder to determine the consumption.

Table 3. Bromatological composition of elephant grass (*Pennisetum purpureum* Schum), utilized as fodder material in the experiment

Items	Months			Mean
	December	January	February	
Dry matter (% of NM)	22.94	25.33	24.74	24.34
Organic matter ¹	96.20	95.76	95.04	95.67
Crude protein ¹	15.14	10.28	9.71	11.71
Neutral detergent fiber ¹	78.29	76.78	83.83	79.63
Indigestible neutral detergent fiber ¹	25.08	25.88	26.11	25.69
Ether extract ¹	2.79	3.29	1.97	2.68
Non-fibrous carbohydrates ¹	4.19	1.61	4.51	3.43
Total carbohydrates ¹	78.27	83.19	83.60	81.69
Mineral matter ¹	3.80	4.22	4.80	4.27

¹% expressed in % of dry matter; NM: Natural matter.

On the 14th day of each experimental period, the behavior of the animals was evaluated, by a single observer, by individual visualization of each animal, for quantification

of the time spent in the following daily activities: fodder intake, rest, rumination and water intake. The behavioral evaluations were carried out for 24 hours, divided into four periods, with 10-minute intervals between them, for a total of 144 observations each 24 hours. These predetermined observation periods were: 06:00 to 09:50 h; 10:00 to 13:50 h; 14:00 to 17:50 h; and 18:00 to 05:50 h), because the period of the day was considered to have an important effect on the behavioral response variables. Finally, the total time spent in each activity was calculated by multiplying the total number of observations by 10 minutes (interval between two observations), and the results were expressed in minutes per day (FISCHER et al., 1998).

On the last three days of each experimental period (12th, 13th and 14th), aliquots of fodder and supplement were collected, for a total of six samples per animal per period. On those same days, fecal samples were collected directly from the rectum of each animal to estimate total excretion, via measurement of indigestible neutral detergent fiber (NDFi), always at 07:00 and 17:00 hours. Finally, on those days, at 07:00 hours samples were collected of the leftover fodder that had been supplied the previous afternoon, and at 17:00 of the leftover fodder supplied in the morning. All the samples collected were immediately frozen at -20 °C for subsequent laboratory analysis.

The samples of the initial and leftover fodder, feces and supplements were analyzed in the Animal Nutrition Laboratory of ICSEZ/UFAM, to determine the following variables: dry matter (DM); crude protein (CP); mineral matter (MM); neutral detergent fiber (NDF); acid detergent fiber (ADF); ether extract (EE); and neutral detergent insoluble nitrogen (NDIN); measured according to the techniques described by Silva and Queiroz (2002).

The concentrations of indigestible neutral detergent fiber (NDFi) were measured in the Animal Nutrition Laboratory of Mato Grosso Federal University, in Cuiabá, MT, by determining *in situ* digestibility (Casali et al., 2008). The organic matter (OM) was estimated by the difference between 100 and the percentage of mineral matter (MM), according to equation 1:

$$\text{Equation 1: } \text{OM (\%DM)} = 100 - \text{MM (\%DM)}$$

The contents of total carbohydrates (TC) and total digestible nutrients (TDN) were estimated via equations 2 and 3 respectively, as proposed by Sniffen et al. (1992):

$$\text{Equation 2: } \text{TC (\%DM)} = \{100 - [\text{CP (\%DM)} + \text{EE (\%DM)} + \text{MM (\%DM)}]\}$$

$$\text{Equation 3: } \text{TDN (g/day)} = \{(\text{CP ingested} - \text{CP feces}) + (\text{TC ingested} - \text{TC feces}) + [2.25 * (\text{EE ingested} - \text{EE feces})]\}$$

The non-fibrous carbohydrates (NFC) from the fodder, leftovers and feces were estimated by equation 4, proposed by Sniffen et al. (1992):

$$\text{Equation 4: } \text{NFC (\%DM)} = \{100 - [\text{CP (\%DM)} + \text{EE (\%DM)} + \text{MM (\%DM)} + \text{NDF (\%DM)}]\}$$

The non-fibrous carbohydrates (NFC) from the supplement were estimated by equation 5, proposed by Hall (2000):

$$\text{Equation 5: } \text{NFC} = 100 - [(\% \text{CP} - \% \text{CP}_{\text{urea}} + \% \text{urea}) + \% \text{NDF} + \% \text{EE} + \% \text{MM}]$$

The fecal excretion was estimated by considering the indigestible neutral detergent fiber (NDFi) as an internal indicator, according to equation 6:

$$\text{Equation 6: } \text{FE}_{(\text{kg DM/day})} = (\text{NDFi consumed}_{(\text{g})} / \text{NDFi fecal}_{(\text{g/kg})})$$

Where:

$$\text{NDFi Consumed} = \text{NDFi ingested (kg/day)}$$

$$\text{NDFi Fecal} = \text{Concentration of NDFi in the feces (g/kgDM)}$$

The total dry matter intake (TDMI) and nutrient dry matter (DMnut) were estimated according to AOAC (1990), by the difference between the quantity of feed supplied and the quantity left over, according to equations 7 and 8:

$$\text{Equation 7: } \text{DMI (kg/day)} = (\text{DRY MATTER OFFERED}_{(\text{kg})} - \text{DRY MATTER LEFT OVER}_{(\text{kg})})$$

$$\text{Equation 8: } \text{DMnut (\%)} = [\text{DMingested} * \% \text{nutrient}] - (\text{Wleftovers} * \% \text{nutrient})$$

The coefficients of apparent digestibility of nutrients (ADN) were estimated by equation 9, according to Maynard et al. (1984):

$$\text{Equation 9: } \text{ADN(\%)} = [\text{DMingested} * \% \text{nutrient}] - (\text{DMexcreted} * \% \text{nutrient}) / (\text{DMingested} * \% \text{nutrient}) * 100$$

The statistical analyses were carried out with a 5x5 Latin square experimental design, composed of five animals, five treatments and five evaluation periods, according to the following statistical model:

$$y_{ijk} = \mu + A_i + \beta_j + P_k + e_{ijk}$$

Where: μ = general constant; A_i = effect of supplement i ($i = 1, 2, 3, 4$ and 5); β_j = effect of animal or treatment sequence j ($j = 1, 2, 3, 4$ and 5); P_k = effect of evaluation period k ($k = 1, 2, 3, 4$ and 5); and e_{ijk} = random error, associated with each observation, assuming normal distribution $(0, \delta^2)$.

The data were analyzed with the PROC MIXED routine of the SAS® statistical package (Statistical Analysis System), version 9.0 for Windows® (2000). All the datasets were tested for normal distribution of errors. When the assumptions were not satisfied, the data were transformed and subsequently retransformed for presentation in the tables. For that purpose, residual normalization was applied, so that each observation (datum) was subtracted from the general mean and divided by the variance. The means of the treatments were

estimated using the LSMEANS tool and comparison was performed via orthogonal contrasts. These contrasts were: protein supplement vs. mineral mixture; and linear and quadratic effect of the levels of protein supplementation.

With respect to animal behavior, the data were analyzed as repeated measures in time, using the PROC MIXED routine of the SAS package. The best covariance structure, determined according to the lowest AIC value (Akaike information criterion), was unstructured (UN) with variance components (VC). When there was no interaction between the levels of supplementation and evaluation time, the factors were evaluated separately. In case of significance between the supplementation levels, the means were compared by contrasting orthogonals. Finally, in the case of evaluation time, the means were compared by the Tukey test at 5% significance.

RESULTS AND DISCUSSION

[Table 4](#) reports the mean values of intake of dry matter and nutrients of the sheep fed on elephant grass and receiving rising concentrations of protein supplementation or only the mineral mixture.

For the variables total dry matter intake (TDMI), total organic matter intake (TOMI), total ether extract intake (TEEI) and total carbohydrates intake (TCI), the protein supplement x mineral mixture contrast was not significant ($p>0.05$), but it was significant ($p<0.05$) for the variables forage dry matter intake (FDMI), total crude protein intake (TCPI), total mineral matter intake (TMMI) and non-fibrous carbohydrates intake (NFICI), demonstrating the possibility of substituting the supplement in place of the fodder. This is confirmed by the results presented in the central part of [Table 4](#), indicating no effect of the contrast on the consumption of specific nutrients from the forage material.

The protein supplement at levels of 100, 150 and 200 g/day gradually increased ($P=0.45$) the TDMI above that attained by the animals in the control group (0.945 kg/day), with values on the order of 0.951, 0.999 and 1.016 kg/day, respectively. However, the animals that received the lowest supplement level (50 g/day) presented the lowest TDMI (0.881 kg/day).

In a similar experiment, Ribeiro et al. (2014) evaluated the intake of forage and performance of lambs weighing 31.80 ± 1.15 kg maintained in an Aruana grass pasture and submitted to rising percentages of crude protein (CP) in the supplement (0, 15, 20, 25 and 30%). The animals received supplement in the amount of 1% of body weight. The authors observed that the increase of crude protein influenced the total consumption of dry matter (kg/day) and the percentage of live weight, with maximum values estimated at 1.296 g (3.2% of DM) with 21.48 and 21.89% of CP in the supplement, respectively. However, the dry matter

Table 4. Mean consumption of dry matter (DM) and nutrients for sheep from consuming tropical forage, receiving rising levels of protein supplementation (50, 100, 150 and 200 g/d) or only mineral mixture

Variables ¹	Mineral mixture	Levels of protein supplementation (g/day)				Contrast ²	Contrast ²				SEM ³
		50	100	150	200		PSxMM	L	Q	C	
Consumption of DM and nutrients in the diet (kg/day)											
FDMI	0.945	0.838	0.865	0.871	0.846	0.001	0.748	0.215	0.929	0.116	
SDMI	-	0.043	0.086	0.128	0.171	-	-	-	-	-	
TDMI	0.945	0.881	0.951	0.999	1.016	0.45	0.001	0.221	0.932	0.116	
TCPI	0.102	0.106	0.119	0.133	0.143	0.001	0.001	0.357	0.685	0.012	
TOMI	0.902	0.837	0.9	0.943	0.956	0.742	0.001	0.204	0.904	0.11	
TMMI	0.042	0.042	0.049	0.054	0.060	0.001	0.001	0.524	0.457	0.007	
TNDFI	0.779	0.694	0.725	0.734	0.722	0.006	0.235	0.224	0.982	0.1	
TEEI	0.023	0.023	0.024	0.027	0.026	0.062	0.001	0.012	0.072	0.003	
TCI	0.780	0.713	0.762	0.788	0.792	0.36	0.003	0.202	0.996	0.099	
NFCI	0.032	0.048	0.068	0.084	0.104	0.001	0.001	0.74	0.334	0.01	
Consumption of DM and nutrients in forage (kg/day)											
FCPI	0.102	0.092	0.094	0.095	0.091	0.006	0.678	0.373	0.639	0.012	
FOMI	0.903	0.8	0.826	0.832	0.807	0.001	0.752	0.21	0.895	0.11	
FMMI	0.042	0.037	0.038	0.038	0.038	0.006	0.578	0.587	0.598	0.007	
FNDFI	0.779	0.688	0.712	0.715	0.697	0.001	0.707	0.228	0.987	0.099	
FEEI	0.024	0.022	0.022	0.022	0.021	0.024	0.745	0.104	0.183	0.003	
FTCI	0.781	0.69	0.715	0.718	0.699	0.001	0.677	0.205	0.981	0.099	
FNFCI	0.032	0.029	0.031	0.029	0.031	0.381	0.813	0.713	0.308	0.009	
Consumption of DM and nutrients in the diet (%BW)											
FDMI	3.695	3.32	3.337	3.536	3.285	0.018	0.846	0.242	0.217	0.339	
SDMI	-	0.173	0.349	0.519	0.716	0.001	0.001	0.8	0.836	0.037	
TDMI	3.694	3.493	3.686	4.057	3.993	0.411	0.004	0.309	0.28	0.337	
FCPI	0.406	0.365	0.368	0.391	0.364	0.015	0.711	0.21	0.174	0.05	
SCPI	0	0.052	0.105	0.157	0.215	0.001	0.001	0.818	0.83	0.011	
TCPI	0.406	0.418	0.473	0.55	0.596	0.001	0.001	0.744	0.36	0.055	
FNDFI	3.034	2.726	2.74	2.905	2.693	0.019	0.871	0.235	0.219	0.286	
SNDFI	0	0.026	0.052	0.078	0.106	0.001	0.001	0.804	0.871	0.006	
TNDFI	3.034	2.751	2.792	2.982	2.799	0.066	0.431	0.246	0.23	0.285	

¹FDMI: forage dry matter intake; SDMI: supplement dry matter intake; TDMI: total dry matter intake; TCPI: total crude protein intake; TOMI: total organic matter intake; TMMI: total mineral matter intake; TNDFI: total neutral detergent fiber intake; TEEI: total ether extract intake; TCI: total carbohydrates intake; TNFCI: total non-fibrous carbohydrates intake; FCPI: forage crude protein intake; FOMI: forage organic matter intake; FMMI: forage mineral matter intake; FNDFI: forage neutral detergent fiber intake; FEEI: forage ether extract intake; FTCI: forage total carbohydrates intake; FNFCI: forage non-fibrous carbohydrates intake; SCPI: supplement crude protein intake; SNDFI: supplement neutral detergent fiber intake. ²Contrasts: PSxMM – protein supplement vs. mineral mixture; L – linear effect; Q – quadratic effect and C – cubic effect. ³SEM: standard error of the mean.

intake from forage was lower among the animals receiving supplement than those only given the mineral mixture. Those results are similar to ours, although with supply of supplement amounts lower than 1% of live weight.

The same authors stated that the associated effects between supplement and forage are determined by the quality of the forage, so that consumption of forage with low quality is not reduced with supply of a supplement, since its ingestion is normally low, but if the forage

has good quality, the supply of supplements can reduce intake of the former, thus causing a substitution effect. The elephant grass used as the voluminous diet component in this study was considered to have medium-high quality, with average of 11.71% CP and 25.69% NDFi ([Table 3](#)) during the experimental period, a condition that fits in the context described.

Similar results were reported by Voltolini et al. (2009), analyzing sheep kept in an irrigated pasture of Tifton 85 grass (high quality) receiving supplement containing protein from different sources (soy meal, urea and cotton seed cake). They observed a reduction of dry matter intake from forage in relation to the non-supplemented animals. However, the authors did not investigate the effect of the treatments on total dry matter intake, as we did.

With respect to consumption of DM and nutrients from the diet, measured as a percentage of body weight (%BW), we found a significant linear effect for the variables supplement dry matter intake (SDMI), total dry matter intake (TDMI), supplement crude protein intake (SCPI), total crude protein intake (TCPI) and supplement neutral detergent fiber intake (SNDFI). The increase in supplement dry matter intake as the levels supplied increased reflected positively on the dry matter intake of the nutrients in the diet.

As shown in [Table 5](#), the substitution of forage grass with the supplement affected the values of apparent dry matter digestibility and nutrients in the diet, and consequently the intake of digestible nutrients. Probably the offer of rising levels of supplement caused an increase of the NFC in the diets ([Table 2](#)), directly influencing the ruminal environment of the supplemented sheep, by increasing the presence of amylolytic bacteria and reducing that of cellulolytic bacteria. This reduced the digestibility of the nutrient fractions composing the NDF.

In this respect, there was a linear effect of the contrast (supplement x mineral mixture) on the apparent DM digestibility ($P=0.02$) and the nutrients, with the exception of the variables AMMD ($P=0.543$) and AEED ($P=0.759$). This was also observed for the consumption of the same variables, DMMI ($P=0.811$) and DEEI ($P=0.291$), besides DTICI ($P=0.195$).

Although the ADMD of the diet declined slightly in numerical terms ($P=0.157$) as the supply of supplement increased, there was a substantial increase of ANFCD ($P=0.002$), DCPI ($P=0.001$) and DNFCI ($P=0.001$). This can be attributed to the greater participation of the supplement in the total diet eaten by the supplemented sheep. According to Costa et al. (2011), protein supplementation increases the efficiency of using forage and increases the retention of nitrogen compounds, and hence enhances digestibility of CP in the gastrointestinal tract compared to the control group (without supplementation).

Those authors also reported that protein supplementation, irrespective of the season of the year, is a priority to optimize forage resources. However, attention should be paid to the level used, since protein is one of the most expensive nutrients in the diet, so that the feeding

Table 5. Mean values of apparent digestibility of nutrients and consumption of digestible nutrients of sheep consuming tropical forage with protein supplementation (50, 100, 150 and 200 g/d) or mineral mixture

Variables ¹	Min- eral mix- ture	Protein supplementation levels (g/day)				Contrast ²				SEM ³
		50	100	150	200	PSxM M	L	Q	C	
Apparent digestibility of nutrients (%)										
ADMD	45.85	46.65	42.98	39.62	38.22	0.157	0.02	0.645	0.879	3.106
ACPD	67.23	71.9	69.48	68.45	65.82	0.456	0.049	0.959	0.742	3.518
AOMD	48.43	48.69	44.71	41.61	39.85	0.09	0.013	0.64	0.966	2.991
AMMD	9.54	12.75	14.3	8.84	12.13	0.414	0.543	0.746	0.202	2.612
ANDFD	48.1	46.11	40.73	35.85	29.75	0.006	0.001	0.896	0.888	2.755
AEED	50.53	47.75	49.22	36.88	54.8	0.64	0.759	0.216	0.143	7.629
ATCD	46.15	45.82	41.29	38.31	35.73	0.092	0.024	0.712	0.93	3.409
ANFCD	52.84	62.7	64.78	70.64	83.28	0.002	0.001	0.154	0.849	9.93
Consumption of digestible nutrients (kg/d)										
DDMI	0.424	0.444	0.4	0.403	0.385	0.327	0.031	0.406	0.332	0.053
DCPI	0.069	0.076	0.082	0.092	0.092	0.001	0.003	0.418	0.394	0.01
DOMI	0.429	0.437	0.394	0.399	0.379	0.084	0.018	0.385	0.232	0.051
DMMI	0.005	0.005	0.007	0.005	0.007	0.408	0.811	1	0.292	0.002
DNDFI	0.37	0.315	0.292	0.267	0.221	0.001	0.001	0.514	0.822	0.044
DEEI	0.012	0.01	0.011	0.009	0.014	0.52	0.291	0.299	0.291	0.002
DTCI	0.353	0.323	0.309	0.306	0.289	0.032	0.195	0.916	0.75	0.047
DNFCI	0.022	0.033	0.046	0.062	0.087	0.001	0.001	0.041	0.617	0.01
TDNI	0.451	0.456	0.417	0.42	0.415	0.097	0.058	0.202	0.376	0.051
Nutritional variables										
TDN (%)	48.65	48.52	44.97	41.58	40.84	0.096	0.026	0.561	0.816	2.703
CP (%)	11.02	12.01	12.94	13.57	14.39	0.001	0.001	0.716	0.457	1.075
CP/TDN	0.231	0.252	0.287	0.332	0.354	0.001	0.001	0.678	0.671	0.026
FE (kg)	0.52	0.474	0.551	0.598	0.63	0.238	0.003	0.502	0.907	0.081
FE (%BW)	2	1.89	2.11	2.39	2.5	0.158	0.005	0.656	0.73	0.222

ADMD: apparent dry matter digestibility; ACPD: apparent crude protein digestibility; AOMD: apparent organic matter digestibility; AMMD: apparent mineral matter digestibility; ANDFD: apparent neutral detergent fiber digestibility; AEED: apparent ether extract digestibility; ATCD: apparent total carbohydrates digestibility; ANFCD: apparent non-fibrous carbohydrates digestibility; DDMI: digestible dry matter intake; DCPI: digestible crude protein intake; DOMI: digestible organic matter intake; DMMI: digestible mineral matter intake; DNDFI: digestible neutral detergent fiber intake; DEEI: digestible ether extract intake; DTCI: digestible total carbohydrate intake; DNFCI: digestible non-fiber carbohydrate intake; TDNI: total digestible nutrients intake; FE: fecal excretion. ²Contrasts: PSxMM - protein supplement vs. mineral mixture; L - linear effect; Q - quadratic effect and C - cubic effect. ³SEM: standard error of the mean.

costs are highly dependent on the efficiency of using protein (BASTOS et al., 2014).

With respect to ingestive behavior, the supply of rising supplementation levels did not affect the daily activities of the sheep; they devoted more time to consumption of forage and supplement near the feeding times (07:00 h and 17:00 h for forage and 10:00 h for supplement). The visual stimulus of the presence of feed material in the respective troughs contributed to this behavior (Table 6).

There was no variation in the time spent drinking water between the treatments. Even at the highest supplement level (200 g/day), the animals were not induced to drink more water due to the greater intake of protein. Besides this, the period spent ruminating and resting coincided with dusk until dawn (18:00 h to 5:50 h), which was expected, since sheep have diurnal habit. According to Sampaio et al. (2016), the time spent ruminating is longer at

Table 6. Eating behavior of sheep consuming tropical forage with protein supplementation (50, 100, 150 and 200 g/d) or mineral mixture, in function of periods of observing behavior (06:00 to 09:50 h; 10:00 to 13:50 h; 14:00 to 17:50h and 18:00 to 05:50h)

Variables	Mineral mixture	Protein Supplementation Levels (g/day)				SEM ¹
		50	100	150	200	
Behavioral results in minutes/day						
Forage consumption time	93.00	98.50	90.50	98.24	90.6	7,12
Supplement consumption time	3.88	3.98	5.03	5.09	4.46	0.07
Water consumption time	2.38	2.63	3.04	2.54	4.03	0.08
Rumination time	131.5	141.5	136.00	131.35	137.4	9,58
Resting time	127.5	110.5	125.00	118.34	132.7	10,99
Observation periods						
	06:00-09:50h	10:00-13:50h	14:00-17:50h	18:00-05:50h		SEM ²
Forage consumption time	138.01 ^a	60.47 ^b	51.67 ^b	126.47 ^a		6.56
Supplement consumption time	2.44 ^b	19.05 ^a	2.63 ^b	3.81 ^b		0.07
Water consumption time	3.17	2.28	3.03	3.1		0.08
Rumination time	43.93 ^c	61.93 ^c	87.93 ^b	348.36 ^a		7.85
Resting time	53.80 ^c	96.60 ^b	96.60 ^b	244.20 ^a		8.83
P-value						
	NS		Time		NS*T	
Forage consumption time	0.87		0.001		0.836	
Supplement consumption time	0.718		0.001		0.959	
Water consumption time	0.279		0.291		0.946	
Rumination time	0.932		0.001		0.547	
Resting time	0.659		0.001		0.968	

¹Standard error of the mean calculated in minutes/day.

²Standard error of the mean calculated in minutes during each observation period. Letters followed by the same letters in the row do not differ by the Tukey test at 5% significance.

NS: non-significant and T: time

night, since the objective of this activity is to reduce the size of the food particles consumed during the day, to favor extraction of nutrients from the diet through processing in the rumen.

CONCLUSIONS

Rising levels of protein supplementation associated with good-quality elephant grass promoted increases of the digestive parameters of the sheep, mainly intake and digestibility of

nutrients. However, the supply of the supplement below 100 g/day resulted in similar parameters to those observed for the mineral mixture.

The presence of the protein supplement in the diet did not significantly affect the intake of water by the sheep, and the ingestive behavior was as expected for the species, with more time spent consuming forage and supplement soon after the predetermined moments of supplying them in the troughs, while rumination and rest occurred mainly after dark.

REFERENCES

- ASSOCIATION OF OFFICIAL ANALYTICAL CHEMISTS - AOAC. **Official methods of analysis of the AOAC**, 15th ed. Arlington, VA, USA: AOAC, 1990.
- BÁNKUTI F.I.; BÁNKUTI S.M.S.; MACEDO F.A.F. A informalidade em sistemas agroindustriais: um estudo exploratório dos hábitos de consumo de carne ovina na cidade de Maringá, Estado do Paraná. **Informações Econômicas**, v.43, p. 5-17, 2013.
- BASTOS, M. P. V.; CARVALHO, G. G. P.; PIRES, A.J.; SILVA, R.R.; FILHO, A.E.; SANTOS, E. J.; CHAGAS, D.M.; BARROSO, D.S.; FILHO, G.A. Ingestive behavior and nitrogen balance of confined Santa Ines lambs fed diets containing soybean hulls. **Asian-Australasian Journal of Animal Sciences**, v. 27, p. 24-29, 2014. <https://doi.org/10.5713/ajas.2013.13076>
- CARVALHO, D.M.G; CABRAL, L.S.; SILVA, J.J.; ABREU, J.G.; GALATI, R.L.; GENTILE, G.G.P.; CAMARGO, L.M.; SOARES, J.Q.; RUFINO JÚNIOR, J. Suplementos para terminação de ovinos em pastos de *Brachiaria brizantha* cv. Marandu. **Revista Semina: Ciências Agrárias**, v. 36, n. 1, p. 313-326, 2015. <https://doi.org/10.5433/1679-0359.2015v36n1p313>
- CARVALHO, D. M. G.; PORTELLA, M. C. L.; MENEZES, L. T.; RUFINO JUNIOR, J.; BRAGA, C. A. S.; TERRES, L.; SOARES, J. Q. Suplementação energética, proteica ou múltipla para ovinos alimentados com forragem tropical de baixa qualidade. **Boletim da Indústria Animal**, Nova Odessa, v.76, p.1-12, 2019. <https://doi.org/10.17523/bia.2019.v76.e1448>
- COSTA, V.A.C.; DETMANN, E.; PAULINO, M.F.; VALADARES FILHO, S.D.C.; HENRIQUES, L.T.; CARVALHO, I.P.C.D.; VALENTE, T.N.P. Consumo e dinâmica ruminal da fibra em detergente neutro em bovinos em pastejo no período das águas suplementados com nitrogênio não-proteico e/ou proteína verdadeira. **Revista Brasileira de Zootecnia**, v.40, p.2805-2814, 2011. <http://dx.doi.org/10.1590/S1516-35982011001200027>.
- FISCHER, V.; DESWYSEN, A.G.; AMOUCHE, E.H.; DUTTILLEUL, P.; LOBATO, J. F. P. Efeitos da pressão de pastejo sobre o padrão nectemeral do comportamento ingestivo de ovinos em pastagem. **Revista Brasileira de Zootecnia**, v.27, n.1, p.164-170, 1998.
- INSTITUTO BRASILEIRO DE GEOGRAFIA E ESTATÍSTICA - IBGE. **Banco de Dados**. 2018. Disponível em:<<https://sidra.ibge.gov.br/tabela/6624#resultado>>. Consulted on: September 5, 2019.
- HALL, M.B. **Calculation of non-structural carbohydrate content of feeds that contain**

- non-protein nitrogen.** Gainesville: University of Florida, 2000. P.A-25 (Bulletin, 339).
- LEITE, E. R.; MEDEIROS, J. X. Agronegócio da Ovinocultura deslanada no Brasil. In: SELAIVE, A. B; OSÓRIO, J. C. S. **Produção de Ovinos no Brasil.** São Paulo: Roca, 2014. p. 563-582.
- MAYNARD, L.A.; LOOSLI, B.S.; HINTZ, H.F.; WARNER, R. G. **Nutrição animal.** 3rd ed. Rio de Janeiro: Freitas Bastos, 1984. 726p.
- RIBEIRO, P.P.; CABRAL, L.S.; ZERVOUDAKIS, J.T.; MIRANDA, L.; ABREU, J. G.; RODRIGUES, R.C.; MORENZ, M.J.F.; OLIVEIRA, I.S.; TOLEDO, C.B.L. Porcentagem de proteína em suplementos para ovinos mantidos em pasto de capim aruana na época seca. **Revista Arquivo Brasileiro de Medicina Veterinária e Zootecnia**, v. 66, p.1779-1786, 2014. <https://doi.org/10.1590/1678-5357>
- SAMPAIO, A.F.; MENDES, F.B.L.; SANTANA JÚNIOR, H.A.; SANTANA, E.O.C.; SILVA, R.R.; SILVA, F.F. Correlação entre comportamento ingestivo e consumo de nutrientes em vacas a pasto. **Revista Científica de Produção Animal**, v. 18, p. 110-120, 2016.
- SERVIÇO BRASILEIRO DE APOIO ÀS MICRO E PEQUENAS EMPRESAS - SEBRAE. 2018. Disponível em: <<http://www.sebraemercados.com.br/ovinocaprinocultura-nobrasil/>>. Consulted on: September 5, 2019.
- SILVA, D.J.; QUEIROZ, A.C. **Análises de alimentos** (métodos químicos e biológicos). 3.ed. Viçosa, MG: Editora UFV, 2002. 235p.
- SNIFFEN, C.I.; O'CONNOR, I.D.; VAN SOEST, P.J. A net carbohydrate and system for evaluating cattle diets: II. Carbohydrate and protein availability. **Journal of Animal Science**, v.70, p.3562-3577, 1992. <https://doi.org/10.2527/1992.70113562x>
- SAS INSTITUTE INC. **SAS/STAT User's Guide**, Version 9.0, Cary, NC: SAS Institute Inc, 2000.
- VOLTOLINI, T.V.; MORAES, S.A.; ARAÚJO, G.G.L.de.; OLIVEIRA, P.L.T.de.; PEREIRA, L.G.R. Urea levels in multiple supplement for lambs grazing on Buffel grass. **Acta Scientiarum**, v.32, p.461- 465, 2010. <https://doi.org/10.4025/actascianimsci.v32i4.7829>