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RUMINANT NUTRITION

Replacing corn silage with extruded forage in sheep feeding

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ABSTRACT. The objective was to evaluate levels of replacement of corn silage with extruded forage (Foragge®) in sheep feeding. Twenty adult Santa Inês ewes in maintenance, and 55.8 kg average weight were housed in metabolic cages, and the treatments consisted of 20, 40, 60 and 80% replacement of corn silage with Foragge® extruded forage with 60% *Uruchloa brizantha* in the diet. The design was completely randomized, with regression analysis and non-parametric analysis at 5% probability. The supply of up to 80% extruded roughage to replace corn silage increased dry matter intake and digestibility, and fecal output in dry matter ($p < 0.05$). Also, it increased the efficiency of ingestion, rumination and chewing activities of sheep, but did not alter urinary parameters and energy metabolites ($p > 0.05$). However, it reduced the concentration of uric acid ($p < 0.05$). Foragge® extruded roughage can replace corn silage by up to 80% with changes in intake, digestibility, fecal parameters, ingestive behavior and concentration of uric acid and urea, with no modifications in urinary parameters and energy metabolites.

Keywords: nutrition; sheep; extrusion.

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Introduction

The most widespread forage conservation methods are haymaking and silage making. Silage is the product resulting from anaerobic fermentation of chopped forage plants and stored in structures called silos (Dantas & Mattos Negrão, 2010). It is an economically produced food with good nutritional value for ruminants, in which corn is the main forage used for ensiling (Dantas & Mattos Negrão, 2010). However, the standard for determining the harvest point is one of the main errors in silage production, and the early or late harvest results in significant losses in total dry matter production and in the percentage of grains in the plant, with these parameters being studied for different hybrids (Klein et al., 2018). Both errors generate losses in silage quality, which can make it feasible to replace corn silage with a product that does not have such difficulties in its production, such as forage obtained through the extrusion process.

Extrusion is a thermomechanical and continuous process whose basic principle is the conversion of a solid material into a fluid mass, through the combination of moisture, heat and pressure. The fluid mass is forced through a die and transformed into a product with predetermined physical and geometric characteristics, thus obtaining the gelatinization of the starch and denaturation of the food protein (Mourão et al., 2012). The extrusion process promotes, in comparison with corn silage for sheep, an increase in the intake and digestibility of starch, proteins and fibers, and also improves food palatability, which are associated with the ease of grasping and the greater availability of nutrients, due to the extrusion process (Oliveira et al., 2018).

The feasibility of using extruded forage has been confirmed in proportions or replacements, with increased dietary digestibility, and maintenance of intake, ingestive behavior, and blood parameters in sheep (Oliveira et al., 2019; Ruela et al., 2020; Silva et al., 2020a). However, the establishment of replacement levels for corn silage, a traditional food in feedlot sheep production, and the possible changes in the intake and digestibility of nutrients, ingestive behavior, as well as the serum concentrations of metabolites are not yet well established.

Therefore, our hypothesis is that the increased use of extruded forage to replace corn silage does not alter dry matter intake and digestibility, ingestive behavior and serum concentrations of metabolites. Therefore, the objective was to evaluate the replacement of corn silage with extruded roughage (Foragge® 60%) on dry matter intake and digestibility, ingestive behavior and metabolites of sheep.

Material and methods

The experiment was conducted at the Capim Branco Experimental Farm, in the goat and sheep sector, Federal University of Uberlândia, during January 2017, with a duration of 21 days, with the first 15 experimental days for the adaptation to the feed and cages, and the last six days, for collection of feces, urine and leftovers of food and water, in addition to blood parameters and ingestive behavior. The experiment was approved by the Ethics Committee on Animal Experimentation (CEUA) under protocol number 092/16.

Twenty Santa Inês non-pregnant, non-lactating ewes in maintenance, with an average body weight of 55.8 kg and aged over three years, were weighed and dewormed with Levamisole (orally) on the first day of the experiment, observing the ocular mucosa. Ewes were later housed in metabolic cages equipped with a feeder, drinker, salt lick, slatted floor and fecal and urine separation equipment, located in a masonry shed.

The treatments consisted of inclusion levels of extruded forage, being tested the product Foragge® 60% (F) replacing corn silage (S). The treatments were: 20% Foragge® and 80% Corn Silage (20%F:80%S); 40% Foragge® and 60% Corn Silage (40%F:60%S); 60% Foragge® and 40% Corn Silage (60%F:40%S) and 80% Foragge® and 20% Corn Silage (80%F:20%S). The diet was provided twice a day, at 08:00 and 16:00h, and animals had free access to water and mineral salt specific for sheep. Foragge® 60% is an extruded product made up of 60% Uro*chloa brizantha* grass, starch, vitamin mineral premix and sunflower meal. The chemical composition of Foragge® 60%, silage and treatments are listed in Table 1.

Table 1. Chemical composition of Foragge® 60%, corn silage and diets according to the treatments.

					Nutrient concentration (g kg^{-1})		
Item	DM	CP	NDF	ADF	TDN	MM	Starch
Foragge® 60% (F)[1]	884.3	70	503.3	333.8	650	44	243
Corn silage (S)[2]	312	80	571.1	361.7	625.2	103	256.3[3]
	Treatments		DM	CP	NDF	ADF	TDN
	20F:80S		426.5	78	557.5	356.1	629.1
	40F:60S		540.9	76	544	350.5	633
	60F:40S		655.4	74	530.4	345	636.9
	80F:20S		769.8	72	516.9	339.4	640.8

DM: Dry matter; CP: Crude Protein; NDF: Neutral Detergent Fiber; ADF: Acid Detergent Fiber; TDN: Total Digestible Nutrients. [1]Values provided by the manufacturer Nutratta®. [2]Values obtained by chemical analysis conducted at the Animal Nutrition Laboratory, Federal University of Uberlândia. [3]Data taken from the Brazilian Food Composition Tables for Cattle, by Valadares Filho, Magalhães, Rocha Júnior and Cappelle (2006).

Diet leftovers were measured daily and whenever a value equal to zero was obtained, the amount offered was increased until reaching a surplus equivalent to 10% of the total supplied. Dry matter intake (DMI) was calculated by the difference between offered and leftovers.

During the collection period, samples of leftovers and feces were taken from each animal; at the end of the period, they were homogenized to a composite sample for further chemical analysis. After the end of the collection period, they were stored at -15°C for preservation. Then, samples were pre-dried (offered, leftovers and feces) in a forced ventilation oven at 65°C for 72 hours. Subsequently, they were ground in a Wiley knife mill, into 1mm particles. Soon after, samples were taken to the laboratory for determination of dry matter of the offered, leftovers and feces, in an oven at 105°C for 24 hours, being then possible to calculate the definitive dry matter according to Maynard, Loosi, Hintz and Warner (1984):

The fecal score was determined daily during the collection period according to the scale proposed by Gomes et al. (2012), in which, on score one (1), feces are dry and lackluster; on scale two (2), feces are normal; on scale three (3), feces are slightly soft; on scale four (4), feces are softened, losing their shape and sticking together (bunch of grapes); on scale five (5), feces are soft and not normally shaped (swine feces); and on scale six (6), feces are diarrheic.

Water was supplied every day in the morning, in plastic buckets, in the amount of six liters per animal; the volume of water was measured in a two-liter plastic beaker accurate to 20 mL, and also in the morning, when necessary (always noting the amount when supplied), and the water leftovers were measured on the following day. A bucket was also used to measure the volume of evaporated water. This control bucket received six liters of water in the morning, and the next morning, the amount of water remaining was measured, and consequently, this value was deducted from the total consumption. The consumption of water offered in the bucket was calculated based on the difference between the offered, the leftovers and the evaporated, and total water consumption was calculated by adding the water in the bucket with the water contained in the ingested food. The water content in the food was obtained by the equation: 100 – DM content of the food. The water requirement of the animal was calculated by the equation proposed by Forbes (1968).

Ingestive behavior was measured on the 5th day of the collection period, in which animals were visually observed by trained people, taking turn, arranged so as not to disturb the animals, for 24 hours, once throughout the collection period. At night, the environment artificially lit, and lights were kept on for five days before the evaluation to promote the animal adaptation. They were checked every five minutes, if the animals were ingesting food or water separately (ING), and if they were in rumination (RUM), idle (IDLE), according to the methodology proposed by Fischer et al. (1998).

Activity calculations were made in minutes per day, assuming that, in the five minutes following each observation, the animal remained in the same activity. The total time spent chewing was determined by adding the times spent in ingestion and rumination. The efficiency of ingestion, chewing and rumination were obtained according to Polli, Restle, Senna and Almeida (1996).

Blood collections to evaluate the biochemical components were made on the first, third and fifth day of the measurement period, always before the first meal, with the fasting animal. To evaluate biochemical components, blood samples were drawn by jugular venipuncture into Vacutainer® tubes without anticoagulant. Biochemical components for determining energy metabolism were: triglycerides and cholesterol; and to determine protein metabolism: total protein (TP), urea, albumin, uric acid and creatinine.

For glycemic assessment, the first collection was performed on day 6 of measurements, at 8 am (before the first meal), 11 am, 2 pm, 5 pm and 8 pm. On the day of glycemic assessment, the second meal was only offered after the 20:00 collection. Samples were collected by jugular venipuncture with the aid of 5 mL Vacutainer® tubes containing fluoride and EDTA, properly identified for each animal. The collected blood samples (glycemia and biochemistry) were centrifuged at 3,000 rotations per minute for 10 minutes, and sera were separated into aliquots, poured into micro tubes and stored in a freezer at -5°C for later laboratory analysis. All samples were processed in a Bioplus 2000 automated biochemical analyzer (Bioplus©, Barueri-SP, Brazil), using a commercial kit from Lab Test (Labtest Diagnóstica S.A., Lagoa Santa-MG, Brazil).

The design used was completely randomized with four treatments and five replications, with statistical analysis according to the model:

$$Y_{ij} = \mu + T_i + e_{ij}$$

where: Y is the observed variable, μ is a constant, T is the fixed treatment effect, and e is the random error.

For blood glucose, repeated measurements in time were taken for the collection times, according to the model:

$$Y_{ijk} = \mu + T_i + P_j + (T * P)_{ij} + e_{ijk};$$

where Y is the observed variable, μ is a constant, T is the treatment fixed effect, P_j is the fixed effect of period, $T*P$ is the interaction between period and treatment, and e is the random error.

For the blood glucose model, it was tested for the sphericity condition, which was not accepted. Therefore, the analysis of mixed models was used, in which all covariance structures (S), autoregressive, unstructured and composite symmetry were evaluated for the dependence of model errors. The covariance structure that best explains the residual correlation was selected using the lowest Akaike information criterion (AICc) (Akaike, 1974).

All data were tested for normality (Shapiro & Wilk, 1965) and homoscedasticity (Levene, 1960) of the variances. Normal variables and those with homogeneous variances were subjected to regression analysis, testing linear and quadratic effects, with a type I error probability and a significance level of 5%. Data referring to the fecal score were analyzed by non-parametric statistics, using the Kruskal and Wallis (1952) test followed by the Conover (1980) procedure with a significance level of 5%. All analyses were performed in the SAS software (2012).

Results and discussion

There was a significant difference for dry matter intake (DMI) in kg day^{-1}, as a function of body weight (%) and as a function of metabolic weight, showing a quadratic behavior ($p < 0.05$). The maximum point for intake was for ewes fed 80% extruded forage and 20% corn silage (Table 2). The minimum point was for 45.33% Forrage® with dry matter intake of 1.12 kg per day. Changes in intake occurred due to forage processing by extrusion providing different particle size and density compared to corn silage, leading to changes in rumen content.

Ruminal match is composed of large particles that are in the process of fermentation by ruminal microorganisms. In the diets, large particles are represented by corn silage, in the treatment in which there is a lower concentration of this, only 20%, the match was not thick, which contributes to the non-occurrence of rumen fill, increasing intake because the rest of the diet consisted of Foragge®, which, according to Oliveira et al. (2018) have very dense 2mm particles that rapidly hydrate and will constitute the rumen pool, where they will be quickly fermented and disappear in the rumen stratification.

This was also reported by Gomes et al. (2012), demonstrating that the reduction in forage particle size, as well as the effect of the extrusion process, increases fiber solubility, increasing the rate of passage of food in the gastrointestinal tract. Thus, by increasing the amount of soluble fiber from the extruded forage by up to 80% in relation to corn silage, there is an increase in the rate of passage and ruminal emptying, and consequently in dry matter intake.

In all treatments, DMI was above the recommended by the Nacional Research Council (NRC, 2007) (Table 2), which implies a greater supply of nutrients to the ewes. According to the NRC (2007), the DMI value in this animal category ranges from 0.91 to 1.05 kg day^{-1}, and the average DMI found was 1.73 kg day^{-1}, which is 76.5% above the recommended, and the average DMI in relation to body weight (DMI%BW) found was 3.11%, with the recommended ranging from 1.75 to 1.83%, that is, 73.74% above the predicted.

Nutrient intake by ruminants is controlled by homeostatic and homoerotic mechanisms, based on nutritional requirements, osmotic control and rumen fill capacity (Allen, 2020). Intake above the recommendation for the category may occur because, maintenance ewes, despite having lower nutritional requirements when compared, for example, to lactating ewes, can maximize dry matter intake when there is no limitation by the repletion effect, coming into positive energy balance and accumulating body reserves (NRC, 2007).

Table 2. Effect of replacing corn silage with extruded forage on dry matter intake and digestibility of ewes.

Treatment	DMI[1](kg day^{-1})	DMI/BW[2](%)	DMI/MW[3]	DDM[4](g kg^{-1})
20% F:80% S	1.48	2.70	73.63	618.1
40% F:60% S	1.51	2.68	73.63	467.6
60% F:40% S	1.39	2.55	69.30	408.5
80% F:20% S	2.53	4.48	123.03	571.1
MG	1.73	3.11	84.90	516.3
CV	15.52	11.60	12.07	12.56
p - value	0.0025	0.0015	0.0002	0.0257

[1]$Y = 2.367355 - 0.054476x + 0.0006x^2$ $R^2 = 88.38\%$; [2]$Y = 4.244650 - 0.095814x + 0.001219x^2$ $R^2 = 90.67\%$; [3]$Y = 116.102245 - 2.639097x + 0.033585x^2$ $R^2 = 90.03\%$; [4]$Y = 95.771340 - 2.056753x + 0.019567x^2$ $R^2 = 96.89\%$; F = Foragge®, CS= Corn silage; GM: general mean; CV; coefficient of variation; MW = metabolic weight (Body weight $^{0.75}$); BW = body weight; DMI: dry matter intake; DDM: digestibility of dry matter.

There was a quadratic behavior for dry matter digestibility ($p < 0.05$), with the highest values for treatments with 80% corn silage and 80% Forrage® in the diet (Table 2), with a minimum point of 401.4 g kg^{-1} for dry matter digestibility with 54.10% Forrage®. The increase in dietary digestibility with the highest percentage of corn silage was due to factors related to intake. With larger particle size and lower density, corn silage had a longer retention time in the rumen environment, for hydration, and the need for particle size reduction for escape. The longer retention time reduces the intake capacity; however, it exposes the digesta to attack by ruminal microorganisms for a longer period, increasing digestibility (Dufreneix, Faverdin, & Peyraud, 2019).

For the treatment with 80% extruded forage, there was an increase in digestibility because, despite the shorter exposure time for degradation compared to the treatment with a higher proportion of corn silage, the higher intake of nutrients was compensatory. Oliveira et al. (2018) reports that the extrusa particle size leads to an increase in the rate of passage, due to the reduction of particles and increase in density in the extrusion, that is, ease of hydration, degradation and escape. Another factor for the increased digestibility is that during the extrusion process, there is starch gelatinization and protein denaturation, facilitating the degradation of the diet (Lancheros, Espinosa, & Stein, 2020).

Values of dry matter digestibility of the treatments with 80% corn silage and 80% extruded roughage were close to the recommended by Valadares Filho et al. (2006) for corn silage of 618.1 g kg^{-1}, demonstrating the ability to replace corn silage, a conventional food, with up to 80% extruded forage, without major losses in the use of nutrients by sheep.

There was no statistical difference in fecal output on a natural matter basis and percentage of fecal dry matter of the ewes with the replacement of corn silage with extruded forage in the diet ($p > 0.05$) (Table 3).

The average for fecal output in natural matter (FNM) was 140% above the recommended by Vieira (2008), from 0.8 to 1.5 kg feces per day, reflecting the average intake of dry matter (Table 2).

Table 3. Effect of replacing corn silage with extruded forage in sheep diet on fecal and urinary parameters and water consumption.

Treatment	FNM	FDMO[3]	FDM	FS*
20F:80S	2.18	0.704	344.4	2.36 AB
40F:60S	2.54	0.861	365.0	2.16 B
60F:40S	3.16	0.935	299.8	2.56 A
80F:20S	3.22	1.168	365.2	2.96 A
GM	2.78	0.917	343.6	2.51
CV	27.50	27.71	28.13	-
P-value	0.4587	0.0365	0.8847	0.0075
Treatment	CH_2O[1]	CH_2O/DMI[2]	Ur. Vol.	Ur. Dens.
20F:80S	1.97	1.34	0.871	1.0282
40F:60S	2.49	1.64	1.130	1.0248
60F:40S	3.31	2.42	1.070	1.0250
80F:20S	3.67	1.43	0.473	1.0396
GM	2.86	1.71	0.888	1.0294
CV	29.95	27.77	32.48	1.16
P-value	0.0241	0.0458	0.7745	0.6912

*Non-parametric statistics; FNM= fecal natural matter (kg dia^{-1}); FDM= fecal dry matter output (g day^{-1}); FDM = fecal dry matter (g kg^{-1}); FS= fecal score; CH_2O= water consumption (L day^{-1}), DMI = dry matter intake (L kg^{-1}), Ur. Vol. = Urine volume (L day^{-1}); Ur. Dens. = Urine density (Ml dL^{-1}); F = Foragge®, CS= Corn silage; MG: general mean; CV: coefficient of variation. [1]$Y = 1.38600 + 0.029600x$; $R^2 = 98.01\%$; [2]$Y = 0.177785 + 0.086534x - 0.000812x^2$; $R^2 = 65.29\%$; [3]$Y = 0.551380 + 0.007325x$; $R^2 = 96.10\%$

For values of fecal dry matter, the value was below the reference for the sheep species, which varies between 370 and 440 g kg^{-1} (Cleef, Ezequiel, D'Aurea, Fávaro, & Sancanari, 2014). This suggests that the feces are more softened due to the higher rate of passage of the diet, a fact demonstrated by the responses in the intake and digestibility of the diets (Table 2). The fecal condition score was different for the proportions of replacement of corn silage wih extruded roughage ($p < 0.05$). According to the fecal score classification, the desirable condition is represented by 2 (Gomes et al., 2012). That is, the average value of the diets was close to normality. However, the treatments with the highest proportion of extruded forage, 60 and 80%, were close to score 3, indicative of soft feces (Table 3). This is because the increase in intake and the maintenance of digestibility close to that of corn silage, combined with the need for water intake to hydrate the particles and the rate of passage, results in feces with higher water content and softened.

As the proportion of extruded forage to replace corn silage in the diet increased, ewe fecal dry matter output (FDMO) increased linearly ($p < 0.05$) (Table 3). Factors related to increased intake and reduced digestibility when replacing silage with extruded forage, that is, lower feed utilization, led to an increase in FDMO.

Water consumption increased linearly with increasing percentage of extruded forage replacing corn silage. According to Oliveira et al. (2018), water consumption is directly related to the increased inclusion of extruded forage due to its high dry matter content, shown with 88% in Table 1. Despite this, water consumption was 49.65% below recommended by Forbes (1968) of 5.68 liters per day, however the animals did not show signs of water stress, reinforcing the need for equations considering tropical regions.

With regard to urine volume and density, there was no significant difference between treatments ($p > 0.05$) (Table 3), which shows that the increase in water intake was to compensate for the lack of water in the extruded forage. The mean value of urine volume was within the recommended range according to Reece (2006), from 100 to 400 mL for every 10 kg body weight for sheep. Likewise, the mean value for urinary density was in agreement with Hendrix (2005), between 1,010 and 1,040 for sheep, demonstrating that sheep had adequate hydration and did not present kidney disorders.

For ingestive behavior, there was no statistical difference for ingestion time ($p > 0.05$) (Table 4). The average ingestion time was above that estimated by Fischer et al. (1997), from one to three hours for sheep. The longer time spent with ingestion was due to the increase in dry matter intake (Table 2).

As for the times of rumination, idle and chewing, there was a significant difference ($p < 0.05$) (Table 4). Rumination time was within the recommended by Fischer et al. (1997), 5.7 hours, with a quadratic behavior, and higher values for treatments with higher percentage of corn silage (Table 4). Corn silage, due to its larger particle size than extruded forage, leads to a greater filling effect, reduction in intake capacity, requiring a greater need for food processing by rumination to escape the rumen environment (Soest, 1994). On the other hand, finely ground or processed foods, such as pelleted or extruded foods, reduce rumination time, due to greater ease of hydration and smaller particle size, which can be observed with increasing inclusion of extruded roughage in the diet (Oliveira et al., 2018).

Table 4. Effect of replacing corn silage with extruded forage in sheep diet on ingestive behavior.

Treatment	Ingestion (min day^{-1})	Rumination[1] (min day^{-1})	Idle[2] (min day^{-1})	Chewing[3] (min day^{-1})
20F:80S	333.00	510.00	597.00	843.00
40F:60S	365.00	527.00	548.00	892.00
60F:40S	284.00	495.00	661.00	779.00
80F:20S	310.00	342.00	788.00	652.00
GM	323.00	468.50	648.50	791.50
CV	22.34	13.28	15.19	12.45
P-value	0.7425	0.0038	0.0003	0.0059
		Ingestive behavior efficiency (g min^{-1})		
Treatment	Ingestion[4]	Rumination[5]	Chewing[6]	
20F:80S	4.55	2.93	1.76	
40F:60S	4.23	2.87	1.70	
60F:40S	5.18	2.84	1.79	
80F:20S	8.36	7.52	3.93	
GM	5.58	4.04	2.30	
CV	20.45	18.65	16.04	
P-value	0.0001	0.0000	0.0000	

F = Foragge®, CS= Corn silage; MG: general mean; CV: coefficient of variation. [1]$Y = 390.00 + 7.9450x – 0.106250x^2$; $R^2 = 98.81\%$; [2]$Y = 477.00 + 3.430x$; $R^2 = 72.69\%$; [3]$Y = 963.00 – 3.430x$; $R^2 = 72.69\%$; [4]$Y = 6.87452 – 0.157315x + 0.002192x^2$; $R^2 = 99.56\%$; [5]$Y = 6.544695 – 0.227925x + 0.02967x^2$; $R^2 = 93.25\%$; [6]$Y = 3.400231 – 0.104508x + 0.001375x^2$; $R^2 = 94.98\%$.

For the same reason, there was a linear reduction in the time spent chewing (Table 4). That is, the diet containing 80% corn silage showed the highest chewing value and, because of that, the increase in DDM (Table 2). This can also be explained by the silage particle size, since, because it is larger, it will remain in the rumen for a longer time, which will increase the action of rumen microorganisms, using dietary nutrients more efficiently (Gomes et al., 2012).

The time spent idle showed an increasing linear behavior ($p > 0.05$), increasing with the higher percentage of use of extruded forage in the diet. According to Fischer et al. (1997), this is inversely related to factors and rumination time.

Regarding the efficiency of ingestion, rumination and chewing, there was a significant difference, in which all variables showed quadratic behavior ($p < 0.05$) (Table 4). The behavior of ingestion, rumination and chewing was effective as the inclusion of extruded forage was increased up to 80% replacing corn silage, because this food is easier to grasp and chew compared to corn silage. Added to these factors, extruded forage has greater solubility and fermentability of nutrients, making the rumination and chewing more efficient (Oliveira et al., 2018).

There was no statistical difference for blood glucose levels when increasing the percentage of replacement of corn silage with extruded roughage (Table 5) ($p > 0.05$). Blood glucose is an indicator of energy balance in animals. The maintenance of glycemia indicates that all treatments had a similar energy supply. The average blood glucose value was within the range recommended by Silva et al. (2020a), for sheep in tropical regions.

Table 5. Effect of extruded forage: corn silage ratio on glucose level.

Treatment	Glycemia (mg dL^{-1})
20F:80S	71.20
40F:60S	73.48
60F:40S	72.40
80F:20S	70.84
GM	71.98
CV	14.66
p - value	0.8632
Reference value*	30 - 94

*Silva et al. (2020b). MG: general mean; CV: coefficient of variation.

For the collection times, blood glucose decreased linearly throughout the day ($p < 0.05$) (Figure 1). This is because, when food is offered, there is a stimulus for having meals (Schultz et al., 2019), thus increasing intake, leading to glucose peaks.

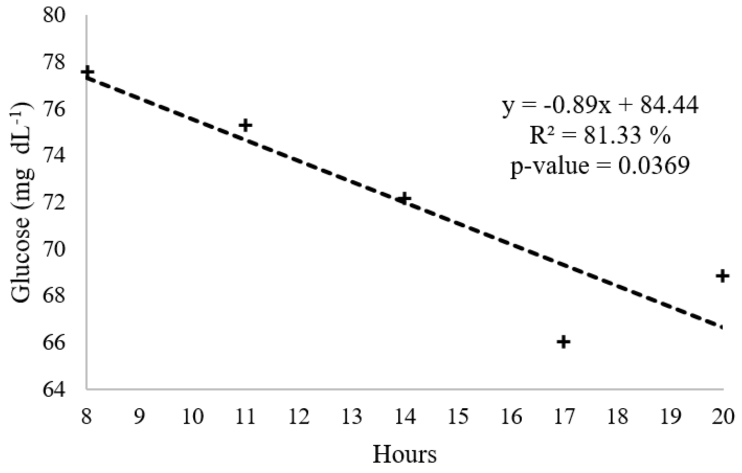

Figure 1. Blood glucose level as a function of collection hours.

The levels of energy metabolites did not differ with increasing percentage of replacement of corn silage with extruded forage (Table 6) ($p > 0.05$). Triglycerides and cholesterol, together with blood glucose, represent the concentration of available energy. Both energy metabolites were within the range recommended by Silva et al. (2020b), showing that despite changes in intake, digestibility, fecal parameters and ingestive behavior, the replacement of corn silage with extruded forage did not change the energy metabolism of sheep.

Table 6. Effect of the extruded forage: corn silage ratio on the concentration of energy and protein metabolites.

Treatment	Triglycerides (mg dL^{-1})	Cholesterol (mg dL^{-1})	Total protein (g dL^{-1})	Albumin (g dL^{-1})	Uric acid[1] (mg dL^{-1})	Urea[2] (mg dL^{-1})	Creatinine (mg dL^{-1})
20F:80S	39.10	37.00	2.78	3.97	0.72A	21.86	0.68
40F:60S	47.20	37.26	2.33	3.81	0.34B	28.46	0.79
60F:40S	37.93	34.06	4.34	3.36	0.39B	39.66	0.86
80F:20S	48.33	35.53	2.48	3.55	0.26B	32.53	0.78
GM	43.14	35.96	2.98	3.67	-	30.63	0.78
CV	14.17	18.75	30.72	15.68	-	18.11	18.77
RV*	5 - 71	14 - 126	3.10 – 10.7	1.1 – 5.2	0.0 – 1.7	10 - 92	0.4 – 1.7
P-value	0.7458	0.8869	0.3648	0.6174	0.0487	0.0087	0.8889

[1]Non-parametric statistics; GM: general mean; [2]$Y= 2,66640 + 1.074348x – 0.008584x^2$; R^2= 84.23%; RV (Reference value, according to Silva et al., 2020b)

For protein metabolites, the replacement of corn silage with extruded forage did not change the serum levels of total proteins, albumin and creatinine ($p > 0.05$). However, it resulted in quadratic behavior for serum levels of urea ($p < 0.05$) (Table 6). The average value of total proteins was 3.87% below the reference value, this even with 20% replacement of corn silage can be, according to Oliveira et al. (2018), due to the higher solubility and denaturation of protein by the extrusion process. Nevertheless, for albumin, uric acid, urea and creatinine values, they were within the recommended range by Silva et al. (2020b), demonstrating that even with reduction in the total protein content, there was no disorder in the protein nutritional status.

Uric acid is related to the amount of microbial protein available for intestinal digestion (Verbic, Chen, MacLeod, & Ørskov, 1990). The reduction in uric acid concentration when replacing corn silage with extruded forage was due to factors related to intake and digestibility of the extruded feed. Although greater starch availability and protein solubility, the extruded forage has a shorter residence time in the rumen, reducing the exposure time by ruminal microorganisms, and consequently reducing the production of microbial protein.

The increase in urea levels with increasing inclusion of extruded forage (Table 6), reiterates that as the rate of passage of the feed through the rumen increases, rumen microorganisms have shorter access to feed, thus protein that reaches the rumen is converted into ammonia and, later, in the liver, it is converted into urea, which is excreted in urine or recycled via saliva (González, 2000).

Conclusion

Foragge® extruded forage can replace corn silage by up to 80%, increasing the intake of dry matter and water, and the dry matter digestibility, in addition to increasing rumination and chewing efficiency without causing any metabolic changes in sheep.

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