SEMINA
Ciências Agrárias

DOI: 10.5433/1679-0359.2025v46n2p443

Feeding behavior and metabolic parameters of lambs fed extruded diets in different roughage:concentrate ratios

Comportamento ingestivo e parâmetros metabólicos de cordeiros alimentados com dietas extrusadas em diferentes proporções de volumoso:concentrado

Karla Alves Oliveira[1]*; Marco Túlio Santos Siqueira[2]; Pedro Henrique Cavalcante Ribeiro[2]; Laylles Costa Araújo[3]; Valdinin Gonçalves de Andrade[4]; Lucas Eduardo Gonçalves Vilaça[5]; Marcela Rodrigues de Oliveira[5]; Luana de Oliveira Faria[6]; Erica Beatriz Schultz[7]; Gilberto de Lima Macedo Junior[8]

Highlights

Different roughage:concentrate ratios did not affect the dry matter intake.
The animals' digestive parameters were similar across experimental diets.
Roughage:concentrate ratios had no effect on blood metabolites in extruded diets.

Abstract

Diets with different proportions of roughage and concentrate influence the feed intake and feeding behavior of ruminants. The use of extruded diets aims to optimize feed efficiency and metabolism, which is essential for improving the productive performance of lambs. The objective was to evaluate the feeding behavior and metabolic parameters of lambs fed different roughage:concentrate ratios on totally extruded diets. Five lambs, 6.0 ± 0.3 months old and weighing 33.3 ± 8.18 kg, were distributed in a Latin square (5 × 5) and fed with different extruded roughage:concentrate (R:C) ratios: 30:70, 40:60, 50:50,

[1] Postdoctoral student in Veterinary Sciences, Universidade Federal de Uberlândia, UFU, Uberlândia, MG, Brazil. E-mail: karla.alves.oliveira@hotmail.com
[2] Doctoral Degree Students in the Postgraduate Program in Animal Science, Universidade Estadual Paulista Júlio de Mesquita Filho, UNESP, Jaboticabal, SP, Brazil. E-mail: marco.s.siqueira@unesp.br; phc.ribeiro@unesp.br
[3] Drª in Animal Science, Universidade Estadual da Região Tocantina do Maranhão, UEMASUL, Imperatriz, MA, Brazil. E-mail: layllesaraujo@gmail.com
[4] Animal Scientist, Universidade Federal de Uberlândia, UFU, Uberlândia, MG, Brazil. E-mail: valdinin.andrade@ufu.br
[5] Master's Degree Student in the Postgraduate Program in Veterinary Sciences, UFU, Uberlândia, Minas Gerais, Brazil. E-mail: lucas.vilaca@ufu.br; marcela.de@ufu.br
[6] Animal Scientist, Universidade Federal de São João del-Rei, UFSJ, São João del-Rei, MG, Brazil. E-mail: luanaolifaria@aluno.ufsj.edu.br
[7] Drª in Animal Science, Universidade Federal de Viçosa, UFV, Viçosa, MG, Brazil. E-mail: erica.schultz@ufv.br
[8] Dr. in Animal Science, UFU, Uberlândia, MG, Brazil. E-mail: gilberto.macedo@ufu.br
* Author for correspondence

Received: July 10, 2024 Approved: Feb. 14, 2025

60:40, and 70:30. The dry matter intake and digestibility (1.47 kg day^{-1} and 67.05%, respectively), water intake (4.71 L day^{-1}), feeding time (169.8 min day−1), rumination time (256.2 min day^{-1}), and metabolic parameters were similar between diets (P≥0.05). The increase in extruded roughage linearly increased the chewing time and linearly reduced idle time (P<0.05). Glucose reached a minimum of 56.4 mg dL^{-1} at approximately 1:20 pm (P<0.05). A roughage ratio between 30:70 and 70:30 in totally extruded diets did not alter the ingestive, metabolic, or behavioral parameters of sheep. Extruded forage can be increased up to 70% in extruded diets for sheep without compromising the intake or digestibility of the diet's dry matter.
Key words: Energy. Extrusion. Fiber. Glucose. Rumination.

Resumo

Dietas com diferentes proporções de volumoso e concentrado influenciam o consumo e o comportamento alimentar dos ruminantes. O uso de dietas extrusadas busca otimizar a eficiência alimentar e o metabolismo, tornando-se essencial para melhorar o desempenho produtivo dos cordeiros. Objetivou-se avaliar o comportamento alimentar e os parâmetros metabólicos de cordeiros alimentados com diferentes proporções de volumoso:concentrado em dietas totalmente extrusadas. Cinco cordeiros, pesando 33,3 ± 8,18 kg e com 6,0 ± 0,3 meses de idade, foram distribuídos em um quadrado latino (5 x 5) e alimentados com diferentes proporções de volumoso:concentrado (V:C) extrusados: 30:70, 40:60, 50:50, 60:40 e 70:30. Consumo de matéria seca e a digestibilidade (1,47 kg dia^{-1} e 67,05%, respectivamente), consumo de água (4,71 L dia^{-1}), tempo de alimentação (169,8 min dia^{-1}), tempo de ruminação (256,2 min dia^{-1}) e os parâmetros metabólicos foram semelhantes entre as dietas (p≥0,05). O aumento do volumoso extrusado aumentou linearmente o tempo de mastigação e reduziu linearmente o tempo ocioso (p<0,05). A glicose atingiu um mínimo de 56,4 mg dL^{-1}, aproximadamente às 13:20 (p<0,05). A relação volumoso:concentrado entre 30:70 e 70:30 em dietas totalmente extrusadas não alterou os parâmetros ingestivos, metabólicos e comportamentais dos ovinos. A forragem extrusada pode ser aumentada em até 70% em dietas extrusadas para ovinos sem comprometer a ingestão e a digestibilidade da matéria seca da dieta.
Palavras-chave: Energia. Extrusão. Fibra. Glicose. Ruminação.

Introduction

High-concentrate diets are used in competitive sheep production systems to maximize body weight gain in a short period of time (Parente et al., 2016). However, they reduce ruminal pH and cause acidosis, compromising an animal's productive performance (Vicente et al., 2022; Zhao et al., 2018). In turn, an increase in the roughage fraction of the diet physically limits food intake in response to slow fiber degradation and rumen filling (Faryabi et al., 2023), resulting in limitations in the growth and body development of ruminants.

In addition to the diet proportion of roughage and concentrate, nutrient intake and animal body performance are affected by the physical form of the food. Although grains are mostly supplied ground, processing, such as extrusion, improves food intake, energy, weight gain, and feed efficiency for ruminants

(Bateman et al., 2009; Ghaffari & Kertz, 2021; Omidi-Mirzaei et al., 2018). However, applying this process to grains increases food costs, considering the high price of grains. In this scenario, increasing the proportion of fiber in the diet improves the economic viability of the system.

Tropical forages are described with high proportions of xylem and sclerenchyma, which are related to a greater time required for chewing and rumination in herbivores (Y. A. Silva et al., 2021b), resulting in longer retention times in the rumen and limitations in the supply of energy and nutrients to animals (Faryabi et al., 2023; Parente et al., 2016). However, the degradation of plant cells, partial breakdown of fibrous structures, and promotion of starch gelatinization (Brand et al., 2023) promoted by extrusion can be understood as a possible technique for reducing the physical limitations to the degradation of roughage foods. The replacement of corn silage up to 80% for extruded roughage increased the dry matter intake and digestibility and the efficiency of ingestion and rumination of sheep but did not alter energy metabolites (D. A. P. Silva et al., 2021a).

Considering the effects of extrusion on fibrous carbohydrates, we hypothesized that in fully extruded diets, sheep can be fed high proportions of roughage without compromising their dry matter intake or behavioral or metabolic parameters. Therefore, the aim of this study was to evaluate the feeding behavior and metabolic parameters of lambs fed different roughage:concentrate ratios in response to the linear increase in forage in fully extruded diets.

Material and Methods

The project was approved by the Comissão de Ética na Utilização de Animais (CEUA) from Universidade Federal de Uberlândia (UFU), under protocol number 092/17, and was conducted at the Sector of Small Ruminants (*Setor de Pequenos Ruminantes* - SEPER/UFU), located in Uberlândia, Minas Gerais, Brasil.

Five non-castrated lambs, ½ Dorper x ½ Santa Inês, with an initial body weight (BW) of 33.3 ± 8.18 kg, 6.0 ± 0.3 months of age, dewormed, and vaccinated against rabies, leptospirosis, clostridiosis, and botulism, were used in this trial. These animals remained individually in metabolic cages for metabolism tests, with dimensions of 1.30 × 0.80 m, equipped with a feeder, drinker, salt shaker, slatted floor, and equipment for separation of feces and urine. They were distributed in a Latin square design (5 × 5), with 5 animals, 5 diets, and 5 evaluation periods consisting of 15 days (10 days to adapting the animals and 5 days for data collection for evaluation periods).

The experimental diets were balanced for the animal category evaluated according to National Research Council [NRC] (2007) for gains of 200 g day^{-1} (Table 1) and corresponded to the increase in extruded roughage: 30:70, 40:60, 50:50, 60:40, and 70:30. The roughage fraction (Foragge®, Nutratta, Itumbiara, Goiás, Brazil; aerial portion from sugar cane - *Saccharum officinarum* + starch, minerals, and urea) and the concentrate (LAC 24®, Nutratta, Itumbiara, Goiás, Brazil) were extruded separately and, subsequently, mixed in the industry according to the different proportions described. The composition and proportion of the ingredients that make up the products were kept confidential, as it is a commercial patent.

Table 1
Chemical composition of experimental foods and diets

Nutrients, % DM	Foods				
	Foragge 2016®		LAC 24®		
Dry Matter*	91.00		91.40		
Crude Protein	7.70		26.81		
Neutral Detergent Fiber	47.80		14.33		
Acid Detergent Fiber	35.20		8.57		
Ether Extract	2.00		1.35		
Mineral Matter	4.70		6.09		
Total Digestible Nutrients	67.00		77.30		
Nutrients, % DM	Experimental diets (R:C)				
	70:30	60:40	50:50	40:60	30:70
Dry Matter*	91.12	91.16	91.20	91.24	91.28
Crude Protein	13.43	15.34	17.26	19.17	21.08
Neutral Detergent Fiber	37.76	34.41	31.07	27.72	24.37
Acid Detergent Fiber	27.21	24.55	21.89	19.22	16.56
Ether Extract	1.81	1.74	1.68	1.61	1.55
Mineral Matter	5.12	5.26	5.40	5.53	5.67
Total Digestible Nutrients	70.09	71.12	72.15	73.18	74.21

*% natural matter (NM); R:C – roughage:concentrate ratio.

Feeding was carried out daily, twice a day at 08:00 and 16:00 with 50% of the total daily food offered in each meal. The food was offered *ad libitum* as a total mixed ratio, allowing leftovers in a range of 5-10% of the total food provided. Leftovers in natural matter (NM) were used to adjust the quantities offered daily. Water and mineral salt were provided *ad libitum* to the animals.

Total feces collection was carried out during the last five days of each experimental period. For that, the cages were cleaned daily in the morning, and all removed material was weighed. For each sheep, 100 g of feces were collected daily, allowing a composite sample (500 g) to each animal at the end of each experimental period. The fecal score (FE) was assessed daily during the collection days, according to Dickson and Jolly (2011): 1 - molded and rigid feces; 2 - slightly uneven and soft stools; 3 - irregular stools, forming a soft pile; 4 - pasty feces, forming a loose pile; and 5 - liquid stools, including diarrhea.

Plastic buckets were attached to the metabolic cages to supply and estimate the animals' ad libitum water intake. The equivalent of 6 L of water was offered daily to each animal early in the morning. The volume of water was monitored visually, and when necessary, additional amounts of water were offered. After 24 h, during the morning shift, residual water was quantified using a volumetric cylinder, and water intake via liquid diet was determined by the difference between the volume offered and the residual and evaporated volumes. Evaporation was quantified daily by the difference between the quantity of water supplied and the residual measured in a bucket installed in the experimental area, protected from access by the animals.

The animals were subjected to visual observation of food and water intake, rumination, and idleness activities on day 15 of each experimental period for 24 h in 5-minute intervals (Fischer et al., 1998). At night, the environment received artificial lighting, with the lambs previously adapted to the light.

The time spent on total chewing (TCT) was determined by summing the feeding (FT) and rumination (RT) times. Feeding (FEf), rumination (REf), and chewing (CEf) efficiencies were determined from the relationship between feeding activities and dry matter intake (Bürger et al., 2000), as follows:

$$FEf = DMI / FT$$

$$REf = DMI / RT$$

$$CEf = DMI / TCT$$

where FEf is the feeding efficiency on DM (g min^{-1}); REf is the rumination efficiency on DM (g min^{-1}); CEf is the chewing efficiency on DM (g min^{-1}); DMI is dry matter intake (g day^{-1}); FT is the feeding time (h day^{-1}); RT is the rumination time (h day^{-1}); and TCT is the total chewing time (h day^{-1}).

Rumen movements were assessed through ruminal auscultation and auscultatory percussion of the abdomen with a stethoscope placed between the 7th and 8th left ribs of the animals at the costochondral junction level (Dirksen et al., 1993). Complete (CRM) and incomplete (IRM) ruminal movements were detected according to the amplitude and strength of ruminal contraction, in which incomplete movements may be indicative of hypomotility. Total ruminal movements were obtained by summing the CRM and IRM. The assessments took place between the 12th and 14th days of each experimental period, early in the morning, and lasted for 5 uninterrupted minutes.

Blood was collected from the animals by venipuncture of the jugular vein with the aid of Vacutainer® tubes (BD, São Paulo, São Paulo, Brazil) on days 11, 13, and 15 of each experimental period to evaluate biochemical components in the blood and on day 15 to evaluate the glycemic curve. Collections took place at 8:00 am, before the first feeding, without prior fasting of the animals, with the last meal offered at 4 pm the previous day. To evaluate the blood glucose curve on day 15, samples were collected at 8:00 am, 11:00 am, 2:00 pm, 5:00 pm, and 8:00 pm, sing tubes with sodium fluoride and ethylenediaminetetraacetic acid (EDTA) as glycolytic inhibitors and anticoagulants, respectively. Collection at regular intervals aimed to capture the glycemic response throughout the day to assess how concentrate levels in the diet dynamically influence blood glucose, facilitating the interpretation of the effects of diet on metabolism rather than specific changes in time. The second meal was offered after the last collection of the night.

The collected blood samples were centrifuged at 3500 rotations per minute (RPM) for 15 min, with the serum separated into aliquots, stored in previously identified 1.5-mL sterile plastic microtubes, and stored in a freezer at −20°C for subsequent laboratory analysis.

The biochemical components for determining the energy profile were triglycerides, cholesterol, fructosamine, and very low density lipoprotein (VLDL, calculated by dividing the triglyceride value by 5) (Friedewald et al., 1972). To determine protein metabolism, total proteins, urea, albumin, uric acid, and creatinine were measured. The enzymatic profile was evaluated using alkaline phosphatase (ALP), aspartate aminotransferase (AST), and gamma-glutamyltransferase (GGT). In the mineral profile, the levels of calcium (Ca), phosphorus (P), and magnesium (Mg) were evaluated. All samples were processed in a Bioplus® 2000 semi-automatic biochemistry (Bioplus Produtos para Laboratório Ltda., Barueri, São Paulo, Brazil), turbidimetry, and an immunology analyzer, using the photoelectric colorimetry method with a commercial kit from Lab Test Diagnóstica S.A.® (Lagoa Santa, Minas Gerais, Brazil).

The food, leftovers, and feces were sampled daily during the collection period to obtain a composite sample for each animal in each evaluation period. All samples were analyzed for dry matter (DM; 105°C), ash (MM; method 942.05) (Association on Official Analytical Chemists [AOAC], 2002), crude protein (CP; AOAC, 1990/954.01), ether extract (EE; method 920.39) (AOAC, 2002), and acidification with hydrochloric acid. The neutral detergent fiber (NDF) was determined according to Van Soest et al. (1991), and acid detergent fiber (FDA) was determined following the method described by Goering and Van Soest (1970) using sodium sulfite and thermostable amylase. Total digestible nutrients (TDN) were determined using a formula proposed by the NRC (2001).

The Latin square design consisted of five experimental diets, five replications (animals), and five periods, following the proposed model:

$$Yijkl = \mu + Qi + Pj + Fk + Tl + Eijkl$$

where Yijkj is the dependent variable; μ is the mean; Qj is the random effect of a Latin square (i=1–5); Pj is the random effect period (j=1–5); Fk is the random effect of the experimental unit (lambs; k=1–5); Tl is the fixed effect of the R:C ratio in the diet (i=30:70, 40:60, 50:50, 60:40, or 70:30); and Eijkl is the residual error.

The data were analyzed for normality using the Shapiro–Wilk test (Shapiro & Wilk, 1965) and for homoscedasticity of residual variances in the SAEG 9.1 program. Subsequently, the data were subjected to analysis of variance (ANOVA) and regression, with the regressor factor being the percentage of roughage and the regression coefficient being the percentage of concentrate. In this analysis, the significance of the linear and quadratic effects and the non-significance of the lack of adjustment of the model were observed, with the probability of type I error being used for decision making at 5%.

The fecal score variable, being non-parametric, was evaluated using the Kruskal Wallis test (Kruskal & Wallis, 1952), followed by the Conover procedure (Conover, 1980), with a significance level of 5% probability of type I error. The glycemia variable (collection time) was evaluated as a subdivided plot, in which the treatments were in the plots and the harvest times were in the sub-plots (8:00 am, 11:00 am, 2:00 pm, 5:00 pm, and 8:00 pm). A significant tendency was considered at $0.05<P<0.10$.

Results and Discussion

The increase in extruded roughage and the consequent change in the roughage:concentrate ratio of the diets did not affect (P>0.05) the dry matter intake (DMI; kg day^{-1} and %BW) or water intake (IH_2O, L day^{-1}) by the animals or the dry matter digestibility of the diets (DMD, %). Values equivalent to 1.47 kg day^{-1}, 4.71 L day^{-1}, and 67.05% were observed for DMI, IH_2O, and DMD, respectively (Table 2).

Table 2
Dry matter intake (DMI), water intake (IH_2O) and dry matter digestibility (DMD) from extruded diets with different roughage:concentrate ratio offered to lambs

Parameters	Roughage:Concentrate ratio					OA	CV (%)	P-value
	30:70	40:60	50:50	60:40	70:30			
DMI, *kg day^{-1}*	1.74	1.48	1.39	1.44	1.28			
DMI, *%BW*	3.95	3.78	3.79	3.84	3.15	3.70	19.07	0.337
DMI, $BW^{0.75}$	38.80	40.14	40.47	40.65	40.56	40.12	4.17	0.355
IH_2O, L *day^{-1}*	4.24	4.77	4.33	4.69	5.51	4.71	17.31	0.551
$IH2_2O$/DMI	3.39	3.44	3.09	3.13	3.20	3.25	18.31	0.461
DMD, *%*	66.56	67.04	69.18	65.35	67.13	67.05	7.08	0.168

OA: overall average; CV: coefficient of variation.

The dry matter intake (DMI; kg day^{-1} and %BW) observed was descriptively higher than the NRC (2007) recommendations for the evaluated category, which ranged from 1.0 to 1.2 kg day^{-1} and 3.51%BW, respectively. Although these results showed no statistical significance, they suggest a tendency toward increased intake. Additionally, the ratio of water intake to dry matter intake (IH_2O) remained within the recommended range of 2–3:1 (NRC, 2007), in which IH_2O accounts for liquid water intake, water contained in feed, and metabolic water from nutrient catabolism (Esminger et al., 1990). Thus, diets high in dry matter, such as extruded feed (over 90% DM; Table 1), may promote an increase in DMI and a proportional response to water intake, supporting the maintenance of the IH_2O ratio observed in this study.

Diets with higher forage levels and consequently a lower proportion of concentrate result in a reduction in DMI (Faryabi et al., 2023; Parente et al., 2016; Pereira et al., 2024) in response to high NDF intake, causing filling and physical limitations of food intake by ruminants (Pereira et al., 2024). As a result, ruminants can use the increase in digesta retention time to compensate for the rate of fiber digestion, increasing the fraction of potentially digestible NDF, NDF digestibility, and the diet (Pereira et al., 2024). However, the present study showed similarities in DMI and DMD, agreeing with the results of Teixeira et al.

(2021) and Pereira et al. (2024), who showed that nutrient digestibility was modulated by changes in food consumption.

Although this study does not provide comparative results with non-extruded diets, previous research has extensively examined the effects of both extruded and non-extruded diets on intake and digestibility parameters (Araújo et al., 2021; Oliveira et al., 2022; Rodrigues et al., 2022; D. A. P. Silva et al., 2021a; D. A. P. Silva et al., 2022; Siqueira et al., 2022; Vilaça et al., 2023). These studies offer a solid foundation in the literature, illustrating how diet processing can influence these parameters. Evaluating diets with forage:concentrate ratios equivalent to 30:70 and 70:30, similar to the diets described in our experiment, Pereira et al. (2024) described a reduction in DMI and DMD for diets with a higher roughage proportion. Therefore, considering the effects of extrusion on the reduction in the structural stability of fibrous components (Brand et al., 2023), it is possible to infer that the similarity in DMI and DMD in the present study is directly associated with the extrusion of the total diet, especially the bulky fraction of the diet. However, there is a need for studies that describe the direct comparison of the different roughage:concentrate ratios associated with extruded and non-extruded diets. The similarities of DMI, DMD, and possibly, the passage rate cause similar behavior for the digesta transported to the upper intestine of the animals and, consequently, for fecal production.

Animals' digestive parameters were similar between the experimental diets (P>0.05; Table 3). Fecal production (expressed in natural matter and DM) corresponded to 0.47 kg of DM day^{-1}. The fecal score corresponded to a scale of 2.0, described as slightly uneven and soft feces (Dickson & Jolly, 2011).

Table 3
Digestive parameters of lambs receiving extruded diets with different roughage:concentrate ratio

Parameters	Roughage:Concentrate ratio					OA	CV (%)	P-value
	30:70	40:60	50:50	60:40	70:30			
Feces, *kg NM day*$^{-1}$	1.15	1.24	1.06	1.32	1.50	1.25	23.62	0.522
Feces, *kg DM day*$^{-1}$	0.42	0.46	0.42	0.48	0.57	0.47	22.81	0.424
Feces, *% DM*	38.36	39.47	40.82	36.93	38.25	38.77	11.06	0.904
Fecal escore	2.20	1.80	1.80	2.12	2.08	2.00	-	0.499

NM: natural matter; DM: dry matter; OA: overall average; CV: coefficient of variation.

The time spent by animals in individual feeding (FT) and rumination (RT) activities were not influenced by the different roughage:concentrate ratios (P>0.05; Table 4). However, total chewing time (TCT), which corresponds to the sum of FT and RT, decreased linearly with the increase in roughage in the diet (P=0.02), in contrast to idle time (P<0.01; Table 4). The efficiency (g min^{-1}) of ingestion, rumination, and chewing were similar between diets (P>0.05).

Table 4
Feeding behaviour of lambs receiving extruded diets with different roughage:concentrate ratio

Parameters	Roughage:Concentrate ratio					OA	CV (%)	P-value
	30:70	40:60	50:50	60:40	70:30			
			Activities, *min day^{-1}*					
Feeding	178	144	173	173	181	168.8	14.33	0.135
Rumination	193	248	245	278	317	256.2	24.80	0.152
Idle	1069	1048	1022	989	942	1014	6.01	<0.010[1]
Chewing	371	392	418	451	498	426	14.3	0.024[2]
			Efficiencies, *g min^{-1}*					
Feeding	7.06	10.40	8.23	8.85	9.89	8.87	32.38	0.204
Rumination	8.57	6.38	5.81	5.43	5.96	6.43	32.11	0.182
Chewing	3.66	3.71	3.37	3.32	3.68	3.55	34.98	0.208

OA: overall average; CV: coefficient of variation; 1Ŷ=1170.5-3.13x, R2 = 0.93%; 2Ŷ=269.5+3.13x, R2 = 0.97%.

The extrusion of feed in all diets potentially reduces particle selection by ruminants. Despite the likely reduction in the physical effects of particles, diets with higher levels of extruded roughage showed increased NDF and acid detergent fiber (ADF) content (Table 1), which may encourage greater chewing activity (total chewing time) (Van Soest, 1994) and reduce idle time. Although reducing the idle time can decrease energy expenditure from prolonged chewing and metabolic activity, potentially increasing the energy available for production and weight gain (Missio et al., 2010), it is important to clarify that this study did not directly assess animal performance outcomes to confirm this effect.

The results indicate that varying the roughage:concentrate ratios did not significantly impact (P>0.05) either complete or incomplete rumen movements, maintaining rates at approximately 2.48 and 0.96 movements in 5 min, respectively (Table 5). However, total rumen movements displayed a quadratic response, peaking at 3.90 movements in 5 min with a roughage:concentrate ratio of 48.2:51.8.

Table 5
Rumen movements in 5 minutes of lambs receiving extruded diets with different roughage:concentrate ratio

Parameters	Roughage:Concentrate ratio					OA	CV (%)	P-value
	30:70	40:60	50:50	60:40	70:30			
Complete	2.6	2.4	2.4	2.4	2.6	2.48	30.86	0.895
Incomplete	0.6	1.2	1.6	1.2	0.2	0.96	30.51	0.874
Total	3.2	3.6	4.0	3.6	2.8	3.44	14.24	0.040[1]

OA: overall average; CV: coefficient of variation; [1]$\hat{Y}$= -1.417143 + 0.220571x – 0.002286x^2, R^2 = 0.96%.

This suggests that, although the overall balance of roughage and concentrate does not drastically alter the types of rumen movements, there is an optimal ratio (48.2:51.8) that maximizes the total rumen motility. Increased rumen movements at this ratio can enhance digesta passage and potentially stimulate feed intake and the absorption of short-chain fatty acids, as previously indicated by Minami et al. (2021) and Prado et al. (2022). However, this peak in rumen motility does not appear to disrupt dry matter digestibility or the animals' biochemical profile, likely because the intensity of rumen activity remains within a range that does not overly accelerate digesta flow or strain metabolic processing. In contrast, diets with a higher proportion of roughage (60% extruded roughage) may reduce rumen motility, as the greater presence of fiber, especially ADF, may occupy more space in the rumen, limiting microbial activity and motility within this compartment.

No effects of the roughage:concentrate ratios of the extruded diets were observed on the animals' blood metabolites (P>0.05; Table 6). The energetic metabolites—triglycerides (21.58 mg dL^{-1}), cholesterol (32.62 mg dL^{-1}), very low density lipoprotein (VLDL, 4.32 mg dL^{-1}) and fructosamine (245.96 μmol L^{-1})—remained within the reference range described by Varanis et al. (2021). Protein and energy metabolites were not influenced by treatments and remained within the range of values described by Varanis et al. (2021): urea (40.32 mg dL^{-1}), creatinine (0.97 mg dL^{-1}), albumin (2.17 g dL^{-1}), total protein (3.07 g dL^{-1}), and uric acid (0.56 mg dL^{-1}); for liver enzymes – alkaline phosphatase (ALP, 113.72 U L^{-1}), gamma glutamyl transferase (GGT, 43.58 U L^{-1}), and aspartate aminotransferase (AST, 89.82 U L^{-1}); and mineral metabolites - calcium (7.09 mg dL^{-1}), phosphorus (8.00 mg dL^{-1}), and magnesium (2.04 mg dL^{-1}).

Table 6
Blood metabolite of lambs receiving extruded diets with different roughage:concentrate ratio

Parameters	Roughage:Concentrate ratio					OA	CV (%)	P-value	RV
	30:70	40:60	50:50	60:40	70:30				
Energetic metabolites									
Triglycerides, *mg dL⁻¹*	23.1	22.0	20.4	20.3	22.1	17.58	10.82	0.365	5-78
Cholesterol, *mg dL⁻¹*	27.9	32.6	30.1	38.7	33.8	32.62	28.65	0.264	15-140
VLDL, *mg dL⁻¹*	4.6	4.4	4.1	4.1	4.4	4.32	10.82	0.257	1-17
Fructosamine, *µmol L⁻¹*	244.5	245.1	241.9	247.9	250.4	245.96	3.27	0.245	11-414
Protein metabolites									
Urea, *mg dL⁻¹*	42.0	45.7	43.8	38.8	31.3	40.32	31.84	0.375	13-100
Creatinine, *mg dL⁻¹*	1.46	0.82	0.89	0.87	0.83	0.97	11.80	0.134	0.4-1.8
Albumin, *g dL⁻¹*	3.61	1.90	1.62	1.48	2.20	2.16	35.83	0.174	1.1-5.4
Total Protein, *g dL⁻¹*	0.43	3.71	3.66	3.74	3.80	3.07	9.56	0.297	3.1-11.4
Uric Acid, *mg dL⁻¹*	1.46	0.24	0.50	0.33	0.26	0.59	38.43	0.996	0-2.9
Liver enzymes									
ALP, *U L⁻¹*	111.6	108.9	132.1	111.9	104.1	113.72	21.3	0.580	58-728
GGT, *U L⁻¹*	46.7	41.3	42.7	39.7	47.5	43.58	15.06	0.244	31-154
AST, *U L⁻¹*	83.2	87.0	80.2	106.0	92.7	89.82	18.87	0.329	47-354
Mineral metabolites									
Calcium, *mg dL⁻¹*	7.24	6.73	7.63	7.15	6.68	7.09	19.33	0.784	4.6-14.2
Phosphorus, *mg dL⁻¹*	7.42	7.76	8.26	8.30	8.20	7.99	19.96	0.907	4.2-16.6
Magnesium, *mg dL⁻¹*	2.13	2.06	2.06	2.01	1.92	2.04	18.91	0.909	1.1-4.7

VLDL: very low density lipoprotein; ALP: alkaline phosphatase; GGT: gamma glutamyltransferase; AST: aspartate aminotransferase; OA: overall average; CV: coefficient of variation; RV: reference values proposed by Varanis et al. (2021).

Differences in the roughage:concentrate ratio in diets can promote changes in the nutrient profile, especially protein and carbohydrates, causing changes in the metabolite profile of animals. However, the absence of results describing nutrient intake limits the understanding of the direct effect of the diet and its nutrient concentrations on blood metabolites in the present study. However, all metabolites evaluated in our work were within the reference value proposed by Varanis et al. (2021) for lambs from birth to 1 year of age under tropical farming conditions.

A quadratic effect was observed for the different times of blood glucose assessment in the different roughage:concentrate ratios of the diets, resulting in a minimum glucose of 56.4 mg dL^{-1} around 1:20 pm (Table 7). However, all values remained within the range described by Varanis et al. (2021).

Table 7
Interaction between treatments and colletion time for glucose blood concentration (mg dL^{-1}) in lambs receiving extruded diets with different roughage:concentrate ratio

R:C	Collection time					OA	CV (%)	P-value	RV
	8:00 am	11:00 am	02:00 pm	05:00 pm	08:00 pm				
30:70	60.8	61.2	62.2	58.0	56.2				
40:60	61.4	59.0	63.4	59.2	62.0				
50:50	63.8	64.6	61.2	62.2	68.2	61.83	12.5	0.040[1]	33-98
60:40	62.6	67.6	59.0	64.8	67.4				
70:30	63.6	55.8	53.6	64.8	63.2				

OA: overall average; CV: coefficient of variation; RV: reference values proposed by Varanis et al. (2021). [1]$\hat{Y}$= 92.82095 – 5.46x + 0.204762x^2, R^2 = 0.52%.

In ruminants, glucose is synthesized mainly by gluconeogenesis in the liver using ruminal propionate as a substrate (Varanis et al., 2021), which is enhanced in diets with a higher proportion of concentrate. However, glucose was not influenced by the difference in the roughage:concentrate ratio, possibly in response to the effect of extrusion on fibrous carbohydrates, making the fiber less stable and potentially more available for ruminal degradation and use as an energy substrate. Considering the quadratic curve described for the different times in which blood glucose was assessed, the lowest concentration between 1:00 pm and 2:00 pm describes the evaluation time furthest from the animals' most recent meal, around 8:00 am, since the meal appears to stimulate the supply of substrate to the rumen and organism for glucose synthesis. Therefore, the constant use of glucose by the animals throughout the day after the meal at 8:00 am continuously reduces the concentration of this metabolite until a new peak of substrate supply, to be understood as new food for the animals. At the 5:00 pm assessment, blood glucose showed an increase in concentration. Although no food was provided at 4:00 pm on the day of the glycemic curve assessment, it is inferred that the animals ingested some fraction of the diet left in the trough from the food offered in the morning as a physiological response to the conditioning of the animals to the nutritional management adopted in the study, referring to the times of solid diet provision.

Furthermore, the high glucose concentration early in the morning (8:00 am) before food was offered may indicate that the animals also ate at some point prior to food being offered at this time; therefore, in future studies, evaluating the dynamics of distribution of the animals' ingestion activity may provide explanations for the results of the present study. Macedo et al. (2007) observed that sheep fed with levels of orange pomace replacing corn silage showed high food intake activity between 12:00 am and 6:00 am, a behavior that can increase glucose in the early morning in the animals, as in our study.

Food processing through heat treatments, such as extrusion, can alter the structure of nutritional compounds, often enhancing the availability of nutrients for ruminal fermentation (M. F. L. Silva et al., 2015). Although it is reasonable to suggest that subjecting the roughage component of the diet to extrusion may improve nutrient bioavailability, leading to enhanced synchrony in ruminal fermentation, this inference should be approached with caution. The similarity observed in the concentration of blood metabolites particularly energy compounds, proteins, and liver enzymes suggests good liver health and effective metabolization of substrate intake. However, these claims are not directly supported by specific data from this study. Therefore, further research is warranted to evaluate the effects of different roughage:concentrate ratios in extruded diets, enabling a more precise understanding of their impacts on fermentation efficiency and nutrient utilization.

Conclusion

The roughage:concentrate ratios ranging from 30:70 to 70:30 in fully extruded diets did not affect the ingestive, metabolic, or behavioral parameters of sheep. Furthermore, incorporation of up to 70% extruded forage into sheep diets can maintain normal levels of dry matter digestibility and intake by the animals.

Acknowledgments

We are grateful for the grants provided by Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq) and for the inputs granted by Nutratta Brazil Ltda.

References

Araújo, C. M., Oliveira, K. A., Macedo, G. L., Jr., Silva, S. P., & Silva, D. A. P. (2021). Parâmetros nutricionais e bioquímicos de ovinos consumindo volumoso extrusado com diferentes teores de Uruchloa brizantha em comparação a silagem de milho tradicional. *Caderno de Ciências Agrárias, 12*(1), 1-11. doi: 10.356 99/2447-6218.2020.25810

Association on Official Analytical Chemists (2002). *Official methods of analysis of AOAC international* (16nd ed.). AOAC International

Bateman, H. G., Hill, T. M., & Schlotterbeck, R. L. (2009). Effects of corn processing, particle size, and diet form on performance of calves in bedded pens. *Journal of Dairy Science, 92*(2), 782-789. doi: 10.3168/jds.2008-1242

Brand, T. S., Dreyer, O., & Van Zyl, J. H. C. (2023). Extrusion of canola oilcake meal and sweet lupins on the production performance of Meatmaster lambs under feedlot conditions. *South African Journal of Animal Science, 53*(5), 689-695. doi: 10.4314/sajas.v53i5.08

Bürger, P. J., Pereira, J. C., Queiroz, A. C. de, Silva, J. F. C. da, Valadares, S. de C., Fº., Cecon, P. R., & Casali A. D. P. (2000). Comportamento ingestivo em bezerros holandeses alimentados com dietas contendo diferentes níveis de concentrado. *Revista Brasileira de Zootecnia, 9*(1), 236-242. doi: 10.1590/S1516-359 82000000100031

Conover, W. J. (1980). *Practical nonparametric statistics* (2nd ed.). John Wiley & Sons.

Dickson, H., & Jolly, S. (2011). *National procedures and guidelines for intensive sheep and lamb feeding systems.* Meat and Livestock Australia. https://www.integritysystems.com.au/globalassets/mla-corporate/research-and-development/documents/l.lsm.0022_-_national_procedures_and_guidelinesfor_intensive_sheep_and_lamb_feeding_systems.pdf

Dirksen, G., Gründer, H. D., & Stöber, M. (1993). Sistema digestório. In G. Dirksen, H. D. Gründer, & M. Stöber (Eds.), *Exame clínico dos bovinos* (pp. 166-228). Rio de Janeiro.

Esminger, M. E., Oldfield, J. L., & Heinemann, J. J. (1990). *Feeds and nutrition* (2nd ed.). Esminger Publishing.

Faryabi, R., Mousaie, A., Bahrampour, J., & Barazandeh, A. (2023). The effect of dietary inclusion of Artemisia sieberi leaves on growth performance, feeding behaviors, ruminal fermentation, feed digestibility, and blood hemato-biochemical profile of growing male lambs. *Tropical Animal Health and Production, 55*(1), 1-41. doi: 10.1007/s11250-023-03455-0

Fischer, V., Deswysen, A. G., Dèspres, L., Dutilleul, P., & Lobato, J. F. P. (1998). Padrões nectemerais do comportamento ingestivo de ovinos. *Revista Brasileira de Zootecnia, 27*(2), 362-369. https://www.sbz.org.br/revista/artigos/2021.pdf

Friedewald, W. T., Levy, R. I., & Fredrickson, D. S. (1972). Estimation of the concentration of low-density lipoprotein cholesterol in plasma, without use of the preparative ultracentrifuge. *Clinical Chemistry, 18*(6), 499-502. doi: 10.1093/clinchem/18.6.499

Ghaffari, M. H., & Kertz, A. F. (2021). Review: effects of different forms of calf starters on feed intake and growth rate: a systematic review and Bayesian meta-analysis of studies from 1938 to 2021. *Applied Animal Science, 37*(3), 273-293. doi: 10.15232/aas.2021-02150

Goering, H. K., & Van Soest, P. J. (1970). *Forage fiber analyses (apparatus, reagents, procedures, and some applications).* Agricultural Research Service.

Kruskal, W. H., & Wallis, W. A. (1952). Use of ranks in one-criterion variance analysis. *Journal American Statistical Association, 47*(1), 583-621. doi: 10.1080/01621459.1952.10483441

Macedo, C. A. B., Mizubuti, I. Y., Moreira, F. B., Pereira, E. S., Ribeiro, E. L. A., Rocha, M. A. D., Ramos, B. M. O., Mori, R. M., Pinto, A. P., Alves, T. C., & Casimiro, T. R. (2007). Comportamento ingestivo de ovinos recebendo dietas com diferentes níveis de bagaço de laranja em substituição à silagem de sorgo na ração. *Revista Brasileira de Zootecnia, 36*(6), 1910-1916. doi: 10.1590/S1516-35982007000800027

Minami, N. S., Sousa, R. S., Oliveira, F. L. C., Dias, M. R. B., Cassiano, D. A., Mori, C. S., Minervino, A. H. H., & Ortolani, E. L. (2021). Subacute ruminal acidosis in Zebu cattle: clinical and behavioral aspects. *Animals, 11*(1), 1-14. doi: 10.3390/ani11010021

Missio, R. L., Brondani, I. L., Alves Filho, D. C., Silveira, M. D., Freitas, L. D. S., & Restle, J. (2010). Comportamento ingestivo de tourinhos terminados em confinamento, alimentados com diferentes níveis de concentrado na dieta. *Revista Brasileira de Zootecnia, 39*(7), 1571-1578. https://tinyurl.com/2p6ph9bs

National Research Council (2001). *Nutrient requirements of dairy cattle* (7nd ed.). National Academy Press.

National Research Council (2007). *Nutrient requirements of small ruminants: sheep, goats, cervids, and new world camelids.* The National Academies Press.

Oliveira, K. A., Siqueira, M. T. S., Oliveira, M. R. de, Dutra, T. O., Sousa, L. F., & Macedo, G. D. L., Jr. (2022). Nutritional and metabolic parameters of sheep fed extruded roughage. *Scientia Agraria Paranaensis, 21*(3), 244-251. doi: 10.18188/sap.v21i3.29787

Omidi-Mirzaei, H., Azarfar, A., Kiani, A., Mirzaei, M., & Ghaffari, M. H. (2018). Interaction between the physical forms of starter and forage source on growth performance and blood metabolites of Holstein dairy calves. *Journal of Dairy Science, 101*(7), 6074-6084. doi: 10.3168/jds.2017-13892

Parente, H. N., Parente, M. de O. M., Gomes, R. M. da S., Sodré, W. de J. dos S., Moreira, M. A., Fº., Rodrigues, R. C., Santos, V. L. F. dos, & Araújo, J. dos S. (2016). Increasing levels of concentrate digestibility, performance and ingestive behavior in lambs. *Revista Brasileira de Saúde e Produção Animal, 17*(2), 186-194. doi: 10.1590/S1519-99402016000200006

Pereira, A. L., Parente, M. de O. M., Sousa, E. A. de, Alves, A. A., Zanine, A. de M., Ferreira, D. de J., Perazzo, A. F., Sousa, F. C. da S. de, Anjos, L. F. dos, Lima, H. C., Moreira, M. A., Fº., Cavalcanti, H. S., & Parente, H. N. (2024). Nutritional strategies, performance, digestibility, and carcass traits of Santa Ines and Rabo Largo breeds in a tropical climate. *Tropical Animal Health and Production, 56*(2), 1-13. doi: 10.1007/s11250-024-03921-3

Prado, I. N., Ramos, T. R., Prado, R. M. do, Ornaghi, M. G., Stuani, O. F., & Penha, G. P. (2022). Sara (Subacute Ruminal Acidosis) sobre o desempenho e comportamento de bovinos: revisão. *Pubvet, 16*(6), 1-11. https://tinyurl.com/2ezukk6u

Rodrigues, G. R. D., Siqueira, M. T. S., Oliveira, K. A., Oliveira, M. R., Schultz, E. B., Macedo, G. L., Jr. (2022). Casca de soja extrusada em substituição a silagem de milho - parâmetros nutricionais e bioquímicos em ovinos. *Revista Agraria Academica, 5*(1), 147-162. doi: 10.32406/v5n1/2022/147-162/agrariacad

Shapiro, S. S., & Wilk, M. B. (1965). An analysis of variance test for normality, (complete samples). *Biometrika, 52*(1), 591-611. doi: 10.2307/2333709

Silva, M. F. L., Santos, L., & Choupina, A. (2015). A extrusão em tecnologia alimentar: tipos, vantagens e equipamentos. *Revista de Ciências Agrárias, 38*(1), 3-10. https://bibliotecadigital.ipb.pt/entities/publication/ac99f5fa-2f65-4ca4-95a7-77961f42553b

Silva, D. A. P., Loreno, M. B. N., Schultz, E. B., Siqueira, M. T. S., Oliveira, K. A., & Macedo, G. L., Jr. (2022). Replacing corn silage with extruded forage in sheep feeding. *Acta Scientiarum - Animal Sciences, 45*(1), e57397. doi: 10.4025/actascianimsci.v45i1.57397

Silva, D. A. P., Rodrigues, G. R. D., Siqueira, M. T. S., Schultz, E. B., Oliveira, K. A., & Macedo, G. L., Jr. (2021a). Avaliação da substituição da silagem de milho por volumoso extrusado na dieta de ovinos. *Caderno de Ciências Agrárias, 13*(1), 1-10. doi: 10.35699/2447-6218.2021.32930

Silva, Y. A., Almeida, V. V. S. de, Oliveira, A. C., Fonseca, R. S., Santos, P. dos, Ribeiro, J. do S., Silva, M. J. M. dos S., & Lima, D. M. de, Jr. (2021b). Can roughage: concentrate ratio affect the action of red propolis extract on sheep metabolism? *Tropical Animal Health and Production, 53*(472), 1-10. doi: 10. 1007/s11250-021-02907-9

Siqueira, M. T. S., Oliveira, K. A., Schultz, E. B., Sousa, L. F., Silva, V. R. S., & Macedo, G. L., Jr. (2022). Avaliação do efeito da substituição de silagem de milho por ração extrusada de fibra de cana em ovinos. *Revista Agrária Acadêmica, 5*(1), 163-177. doi: 10.32406/v5n1/2022/163-177/agrariacad

Teixeira, A. B. M., Schuh, B. R. F., Daley, V. L., Pinto, P. H. N., Fernandes, S. R., & Freitas, J. A. de. (2021). Performance, biochemical and physiological parameters of Dorper× Santa Ines lambs fed with three levels of metabolizable energy. *Tropical Animal Health and Production, 53*(1), 1-13. doi: 10.1007/s11250-021-02797-x.

Van Soest, P. J. (1994). *Nutritional ecology of the ruminant* (2nd ed.). Cornell University Press.

Van Soest, P. J., Robertson, J. B., & Lewis, B. A. (1991). Methods for dietary fiber, neutral detergent fiber, and nonstarch polysaccharides in relation to animal nutrition. *Journal of Dairy Science, 74*(10), 3583-3597. doi: 10.3168/jds.S0022-0302(91)78551-2

Varanis, L. F. M., Schultz, E. B., Oliveira, K. A., Sousa, L. F., Cruz, W. F. G., & Macedo, G. de L., Jr. (2021). Serum biochemical reference ranges for lambs from birth to 1 year of age in the tropics. *Semina: Ciências Agrárias, 42*(3), 1725-1740. doi: 10.5433/1679-0359.2021v42n3Supl1p1725

Vicente, A. C. S., Carlis, M. S. de P., Santos, I. J. dos, Silva, A. L. A. da, Dias, P. C. G., Jr., Assis, R. G. de, Sturion, T. U., Biava, J. S., Pires, A. V., & Ferreira, E. M. (2022). Performance, nutritional behavior, and carcass characteristics of feedlot lambs fed diets with non-forage fiber source or sodium bicarbonate. *Tropical Animal Health and Production, 54*(5), 1-12. doi: 10.1007/s11250-022-03297-2

Vilaça, L. E. G., Oliveira, M. R., Freitas, A. B. I. de, Coutinho, C. D. M., Oliveira, K. A., Macedo, G. L., Jr. (2023). Casca de soja extrusada em substituição a silagem de milho: parâmetros nutricionais, bioquímicos comportamento ingestivo de ovino. *Caderno de Ciências Agrárias, 15*(1), 1-11. doi: 10.35699/2447-6218.2023.43883

Zhao, C., Liu, G., Li, X., Guan, Y., Wang, Y., Yuan, X., Sun, G., Wang, Z., & Li, X. (2018). Inflammatory mechanism of Rumenitis in dairy cows with subacute ruminal acidosis. *BMC Veterinary Research, 14*(1), 2-8. doi: 10.1186/s12917-018-1463-7