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Productive, behavioral and metabolic parameters of sheep fed with different roughage: concentrate ratios in fully extruded diets

Parâmetros produtivos, comportamentais e metabólicos de ovinos alimentados com diferentes relações de volumoso: concentrado em dietas totalmente extrusadas

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Highlights

Extruded diets with 34% FDN does not compromise sheep performance and metabolism.
Diets with 30 and 50% extruded roughage alter protein metabolism over time.
Extruded diets with up to 60% roughage do not compromise the liver health of sheep.
Rumination activity does not increase with extruded roughage up to 60%.
In fully extruded diets for sheep, up to 60% roughage may be used.

Abstract

High fiber levels physically limit ruminant intake. However, extrusion increases fiber degradability by decreasing particle stability, and higher proportions of roughages can be inserted into fully extruded diets without compromising production. The objective of this study was to compare the weight gain,

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feeding behavior, and metabolic parameters of sheep given extruded diets with different proportions of roughage and a commercial concentrate. Twenty sheep (eight uncastrated males and twelve females) with 21.30 ± 4.07 kg initial mean body weight and 4.0 ± 0.3 months of age were distributed in randomized blocks and fed fully extruded diets with roughage:concentrate ratios of 30:70, 40:60, 50:50, and 60:40. Body weight and feeding behavior were measured at the beginning and end of 90 days. Blood collection was performed on days 0, 21, 42, 63, and 84. Analysis of variance and regression (roughage level) were used to analyze the data, with an alpha level of 0.5%, and means were compared by a Student-Newman-Keuls test with significance at P≤0.05. The average daily gain (0.263 kg/day; P=0.12), the time spent in food intake (188.5 min/day; P=0.11), rumination (190.5 min/day; P=0.11), and leisure (1,061 min/day; P=0.13), and the serum levels of urea (30.1 mg/dL), uric acid (0.20 mg/dL), albumin (2.19 mg/dL), creatinine (1.04 mg/dL), triglycerides (26.54 mg/dL), very low density lipoprotein (5.67 mg/dL), cholesterol (55.22 mg/dL), gamma-glutamyl transferase (57.44 per UL), and alkaline phosphatase (338.68 per UL) were not influenced (P>0.05) by the roughage portion in the diet. Serum total protein increased linearly (P<0.05) over time in diets with 30 and 50% roughage. Aspartate aminotransferase was superior in diets with 30 and 50% roughage (P=0.02). Serum uric acid, albumin, gamma-glutamyl transferase, and aspartate aminotransferase increased linearly over time, while triglycerides, very low-density lipoprotein, cholesterol, and alkaline phosphatase decreased linearly over time (P<0.05). Up to 60% roughage may be added to fully extruded diets for lambs.
Key words: Feeding behavior. Muscle growth. Energy. Fiber. Metabolites.

Resumo

Níveis elevados de fibra limitam fisicamente o consumo de ruminantes, contudo a extrusão aumenta a degradabilidade da fibra através da diminuição da estabilidade das partículas. É possível que maiores proporções de volumosos possam ser inseridas em dietas totalmente extrusadas, sem comprometer a produção. Objetivou-se avaliar o desempenho, comportamento ingestivo e parâmetros metabólicos de ovinos submetidos a dietas extrusadas com diferentes proporções de volumoso:concentrado. Vinte ovinos (oito machos não castrados e doze fêmeas), com 21,30 ± 4,07 kg de peso corporal inicial e 4,0 ± 0,3 meses de idade foram distribuídos em blocos casualizados e alimentados com dietas totalmente extrusadas em diferentes relações de volumoso:concentrado: 30:70, 40:60, 50:50 e 60:40. O peso corporal e comportamento ingestivo foram aferidos no início e final do estudo. A coleta de sangue foi realizada em d0, d21, d42, d63 e d84, antes do fornecimento da dieta. Os dados foram submetidos a análise de variância e regressão (nível de volumoso) considerando probabilidade de significância em 0,5%. Quando necessário, as médias foram comparadas pelo teste SNK com P≤0,05. Os parâmetros bioquímicos foram analisados no tempo, com significância em P≤0,05 e tendência em 0,05<P≤0,10. O ganho médio diário (0,263 kg d^{-1}; P=0,12), os tempos despendidos em ingestão de alimentos (188,5 min d^{-1}; P=0,11), ruminação (190,46 min d^{-1}; P=0,11) e ócio (1061 min d^{-1}; P=0,13) e os níveis séricos de ureia (30,1 mg dL^{-1}), ácido úrico (ACU; 0,20 mg dL^{-1}), albumina (ALB; 2,19 mg dL^{-1}), creatinina (1,04 mg dL^{-1}), triglicerídeos (TRIG; 26,54 mg dL^{-1}), VLDL (5,67 mg dL^{-1}), colesterol (COL; 55,22 mg dL^{-1}), gama-glutamil transferase (GGT; 57,44 U L^{-1}) e fosfatase alcalina (ALP; 338,68 U L^{-1}) não foram influenciados (P>0,05) pelo incremento de volumoso. A proteína total sérica aumentou linearmente (P<0,05) entre d0 e d84 em dietas com 30 e 50% de volumoso. A aspartato aminotransferase (AST) foi superior em dietas com 30 e

50% de volumoso (P=0,02). O ACU, ALB, GGT e AST séricos aumentaram linearmente entre o d0 e d84, enquanto TRIG, VLDL, COL e ALP reduziram linearmente durante o mesmo período (P<0,05). A relação volumoso:concentrado em dietas totalmente extrusadas não alterou o desempenho e comportamento ingestivo dos ovinos, contudo aumentou a concentração de proteínas totais e AST em 30 e 50% de volumoso, sem ultrapassar o limite para a espécie. O volumoso pode ser inserido em até 60% em dietas totalmente extrusadas para cordeiros.

Palavras-chave: Comportamento ingestivo. Crescimento muscular. Energia. Fibra. Metabólitos.

Introduction

The multicavitary stomach and ruminal colonization by microorganisms that degrade fiber provide ruminant animals with a high capacity for ingesting bulky food. However, the slow degradation and longer retention time of the fiber in the rumen limits the activity of ruminal bacteria and food intake by animals (Silva et al., 2021; Faryabi et al., 2023), compromising the availability of nutrients and productive performance of ruminants (Parente et al., 2016; Moura et al., 2019). In contrast, diets rich in concentrate increase energy supply and reduce slaughter time of animals (Parente et al., 2016), while increasing the risks of metabolic disorders (Zhao et al., 2018). This nutritional scenario requires the development of strategies that increase the efficiency of the nutritional use of fiber by ruminants to ensure the digestive health of animals without limitations in intake and performance. The action of extrusion in decomposing plant cells and partially breaking down the fibrous components of food (Gjorgjievski et al., 2022; Brand et al., 2023; Ghorbani et al., 2020) possibly results in alteration of fiber stability and improvement in the degradation process of bulky food, enhancing the energy supply for animals.

Although the effects of the fiber and concentrate ratio on the production and health of ruminant animals are known (Parente et al., 2016), the use of fully extruded diets with a potential increase in energy supplied to the body triggers the need to identify the roughage:concentrate ratio suitable for sheep to (a) allow high energy intake to meet nutritional requirements, (b) maximize production performance, and (c) guarantee the health and well-being of the animals. These effects can be understood through the biochemical profile of ruminants, given that blood components such as albumin, urea, creatinine, triglycerides, and liver enzymes are strongly affected by the level of energy intake (David et al., 2012; Fernandes et al., 2012; Pereira et al., 2018; Varanis et al., 2021). Variations in these metabolites indicate energy availability in the body and describe changes in the protein and energy metabolism of lambs (Teixeira et al., 2021).

Based on the known the effect of extrusion on food and the influence of energy on animal metabolism, we hypothesized that it is possible to increase the fraction of roughage in fully extruded diets without negatively affecting the production, behavioral, and biochemical parameters of sheep. The objective, therefore, was to evaluate the weight gain, feeding behavior, and serum metabolites of sheep fed fully extruded diets with different roughage:concentrate ratios.

Material and Methods

This project was approved by the Ethics Committee on the Use of Animals of the Federal University of Uberlândia (UFU), under protocol 094/17, and conducted in the Small Ruminants Sector (SEPER)/UFU in Uberlândia, Minas Gerais, Brazil.

Animals, treatments, and experimental food management

Twenty ½ Dorper blood x ½ Santa Inês blood sheep, eight uncastrated males and twelve females, with 21.30 ± 4.07 kg of initial body weight and 4.0 ± 0.3 months of age, dewormed and vaccinated against rabies, leptospirosis, clostridiosis, and botulism, were distributed in a randomized block design (DBC, excluding sex effect) and allocated to four collective stalls provided with a drinking fountain, trough, and salt shaker. The sheep were held in the stalls for 105 days, 15 days for adaptation and 90 days for data collection.

The experimental diets were balanced according to the National Research Council [NRC] (2007) for gains of 150 g/day (Table 1), and incremental increases of extruded roughage created four roughage:concentrate ratios (R:C) of 30:70, 40:60, 50:50, and 60:40. The roughage (Forrage®; aerial part of sugarcane (Saccharum officinarum) plus starch, minerals, and urea) and the concentrate (LAC 24®) were extruded separately and subsequently mixed according to the treatment ratios. The composition and proportions of the constituent ingredients of the registered products are patented and confidential.

Table 1
Chemical composition of foods and experimental diets

Nutritional attributes, (% DM)	Foods	
	Foragge®	LAC 24®
Dry Matter	91.00	91.40
Mineral Matter	4.70	6.09
Organic Matter	95.30	93.91
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
Total Digestible Nutrients	67.00	77.30

Nutritional attributes, (% DM)	Roughage:concentrate ratio			
	30:70	40:60	50:50	60:40
Dry Matter	91.28	91.24	91.20	91.16
Mineral Matter	5.67	5.53	5.40	5.26
Organic Matter	94.33	94.47	94.61	94.74
Crude Protein	21.08	19.17	17.26	15.34
Neutral Detergent Fiber	24.37	27.72	31.07	34.41
Acid Detergent Fiber	16.56	19.22	21.89	24.55
Ether Extract	1.55	1.61	1.68	1.74
Total Digestible Nutrients	74.21	73.18	72.15	71.12

The sheep were fed twice daily at 8:00 am and 4:00 pm, with 50% of the total daily food amount offered in each shift. The animals were fed ad libitum, allowing leftovers in a range of 5–10% of the total feed provided. The leftovers of natural matter were used to adjust the quantities offered daily due to the high dry matter (DM) content of the food in its natural form (Table 1). Mineral salt and water were offered ad libitum.

Body performance and feeding behavior

The body weights of the animals at the beginning and end of 90 days were measured to determine the average daily gain (ADG). The sheep were evaluated twice for feeding behavior by observing the activities of food intake, rumination, and leisure at 5-min intervals during of 24 hr (Fischer et al., 1998) at the beginning and end of the 90 days. The nocturnal evaluation was performed with artificial lighting with fluorescent lights, after adapting the animals for three days prior to the behavioral analysis.

Blood and chemical analyses

Blood samples were taken from the sheep by venipuncture of the jugular vein punctually at 08:00 am before feeding. The blood was collected into a Vacutainer® tube with clot activator on day(d)0, d21, d42, d63, and d84. The tubes were centrifuged (3,500 rpm for 15 min). The serum from each sample was placed in an appropriately marked 1.5-mL, sterile, plastic Eppendorf microtube, and the tubes were stored in a freezer at -20 ºC.

The analyses of the serum were performed in a semi-automatic biochemistry, turbidimetry, and immunology Bioplus® 2000 analyzer, employing the photoelectric colorimetry method using a commercial kit from Lab Test Diagnóstica S.A.®. The biochemical components for determining the energy profile were triglycerides, cholesterol, and very low-density lipoprotein (VLDL) calculated by dividing the triglyceride value by five (Friedewald et al., 1972). To evaluate protein metabolism, total proteins, urea, albumin, uric acid, and creatinine were measured. For the enzymatic profile, alkaline phosphatase (Alp), aspartate aminotransferase (AST), and gamma-glutamyltransferase (GGT) were evaluated. To determine the mineral profile, calcium (Ca), phosphorus (P), and magnesium (Mg) contents were quantified.

Food and leftovers were sampled daily to obtain a composite sample. At the end of 90 days, the samples were homogenized, and 20% of the total content was used for laboratory analysis. DM (dried at 105 ºC), mineral MM; (Association on Official Analytical Chemists, [AOAC], 2002) [method 942.05]), crude protein (PB; AOAC, 2002, 1990/954.01), ether extract by acid hydrolysis using petroleum ether (EE; AOAC, 2002; method 920.39), and acidification with hydrochloric acid were measured. Neutral detergent fiber (NDF) was obtained according to Van Soest et al. (1991) and acid acid detergent (FDA) according to Goering and Van Soest (1970) by using sodium sulfite and thermostable amylase. Total digestible nutrients (TDN) were obtained using the formula proposed by the NRC (2001).

Statistical analysis

Data were analyzed using SAEG 9.1. The experimental statistical DBC model, excluding sex effect, considered four levels of extruded roughage in fully extruded diets:

$$Y_{ij} = \mu + A_i + E_{ij}$$

where Yij = dependent variable, μ = overall mean, Ai = fixed effect of R:C diet, and Eklj = residual error.

The data were subjected to a normality test of the residues by the Shapiro Wilk test and of the homogeneity of the variances by Levene's test. Subsequently, the results were subjected to an analysis of variance by the F test with significance at P≤0.05 and regression analysis – the regression factor being the percentage of roughage and the percentage of concentrate as the regression coefficient – for linear and quadratic effects and non-significance of the lack of adjustment of the model, with the probability of type I error at 5%. Treatment means were

compared by a Student-Newman-Keuls test with P≤0.05 for type I error and trend at 0.05<P≤0.10. Biochemical parameters were evaluated as a repeated measure over time, considering the level of extruded roughage in the diet as a plot and the evaluation days (d0, d21, d42, d63, and d84) as a subdivided plot, considering significance at P≤0.05 and trend at 0.05<P≤0.10.

Results

Roughage increment does not compromise performance and feeding behavior of lambs fed fully extruded diets

There was no effect of diet R:C on ADG (262.67 g/day; P = 0.12). The times spent by the lambs in food intake (188.52 min/day), rumination (190.46 min/day), and leisure (1,061.02 min/day) were similar (P>0.05) among the different R:C (Table 2).

Table 2
Average daily gain and feeding behavior of lambs fed extruded diets at different roughage:concentrate ratios

Parameter	Roughage:concentrate ratio				CV (%)	P-value
	30:70	40:60	50:50	60:40		
Average Daily Gain, kg	258.81	256.51	239.65	295.69	13.73	0.12
Feeding Time, min d^{-1}	205.00	192.08	182.00	175.00	10.40	0.11
Rumination Time, min d^{-1}	199.58	168.75	173.00	220.50	28.08	0.11
Leisure Time, min d^{-1}	1035.42	1079.17	1085.00	1044.50	25.11	0.13

CV = coefficient of variation.

The evaluation day increased the protein metabolites concentration in sheep fed extruded diets in different ratios of roughage:concentrate

The serum metabolites evaluated, regardless of the diet R:C and the day of evaluation, were within the reference range described by Varanis et al. (2021) for sheep from birth to one year of age in the tropics.

The R:C did not affect the concentrations of urea, uric acid, albumin, creatinine, and Mg (P>0.05). However, the serum Ca level decreased linearly (P=0.01) in response to the increase in the proportion of roughage (Figure 1), with a reduction of 23.53% between the diets with 30 and 60% roughage. Serum uric acid, albumin, and Mg increased linearly (P<0.05) over time, with increases of 107.7, 83.44 and 53.94%, respectively, between d0 and d84. Creatinine levels over time tended (P = 0.06) to follow a quadratic model, with an estimated maximum of 1.15 mg/dL on d39, an increase of 27.78% over the level on d0.

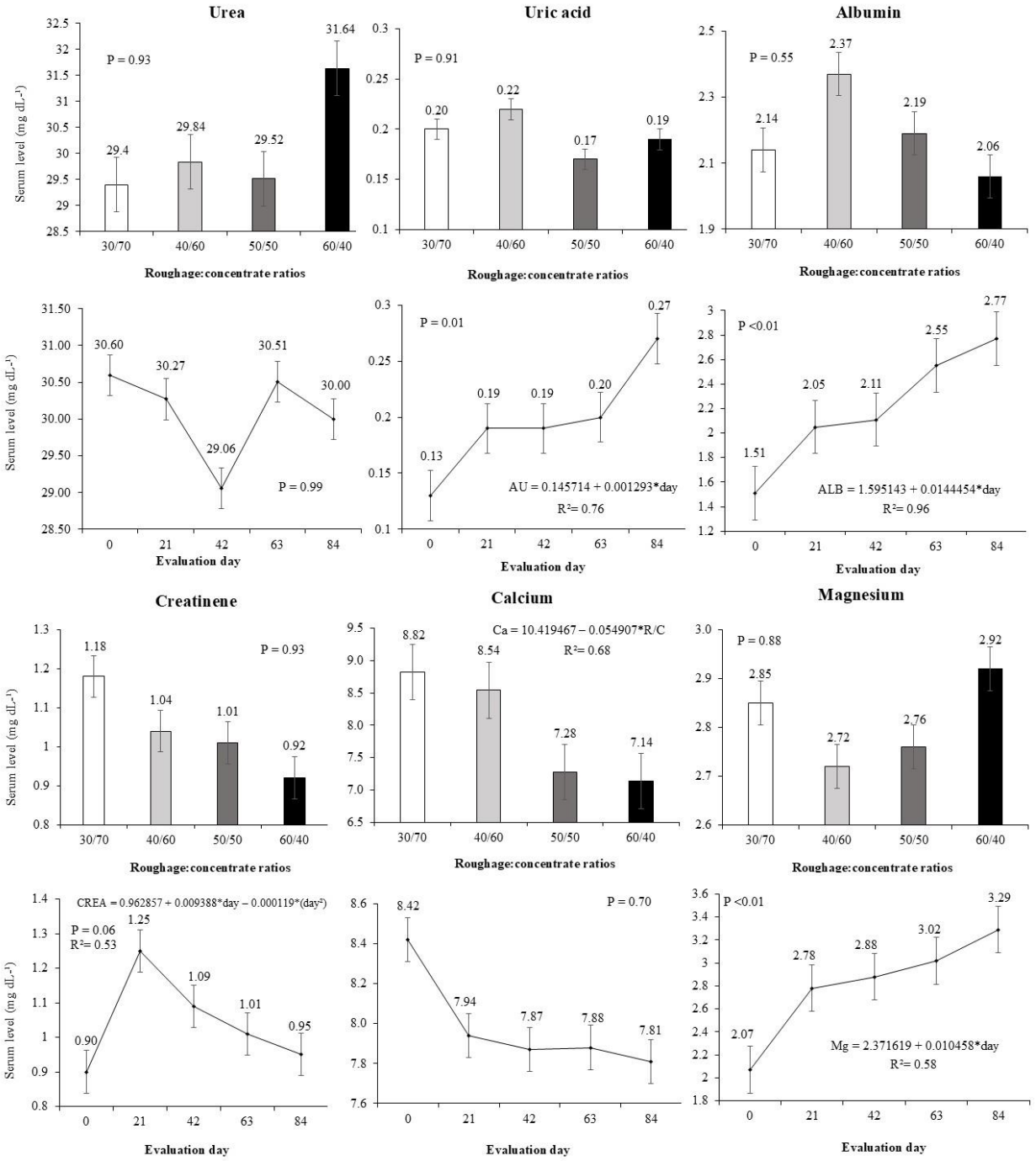

Figure 1. Protein and mineral profile of lambs fed extruded diets with different roughage: concentrate ratios and evaluation times.

The concentrations of total protein and P showed an interaction effect between R:C and evaluation day (Figure 2). From d0 to d84, total protein increased (P < 0.05) by 65.11% (7.08 to 11.69 g/dL) in the 30:70 diet and by 44.52% (7.12 to 10.29 g/dL) in the 50:50 diet. The concentration of P decreased linearly ducing P in diets by 41.63% (8.36 to 5.98 mg/dL) in the 50:50 diet and by 39.65% (7.92 to 4.78 mg/dL) in the 60:40 diet. The P concentration over time in sheep fed the 30:70 diet followed a quadratic model (P<0.01), with a minimum of 1.08 mg/dL on approximately d45 (Figure 2), representing reductions of 86.96% on d0 and 84.53% on d84.

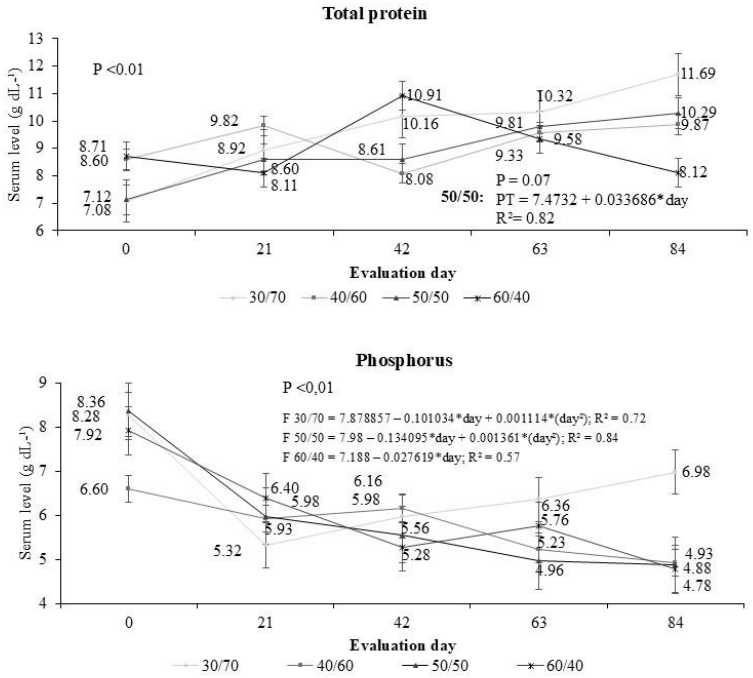

Figure 2. Interaction between roughage: concentrate ratio and evaluation period for total protein and phosphorus in lambs fed extruded diets.

Roughage:concentrate ratio and evaluation day increased liver enzyme concentration and reduced energy metabolites in sheep fed extruded diets

Triglycerides, cholesterol, and VLDL were not significantly (P>0.05) influenced by the R:C (Figure 3), but they decreased linearly (P<0.05) over time by 48.02, 29.47, and 45.10%, respectively. AST was affected by diet composition, with increases of 22.79% and 40.60% in sheep fed the diets with 30 and 50% roughage, respectively. AST increased linearly (P<0.05) by 94.51% from d0 to d84. Concentrations of GGT and Alp over time followed a quadratic model. The estimated minimum GGT was 32.92 per UL, 35.02% lower than on d0 and 60.54% lower than on d84. The estimated minimum Alp 284.14 per UL, 37.97% lower than on d0 and 11.28% lower than on d84.

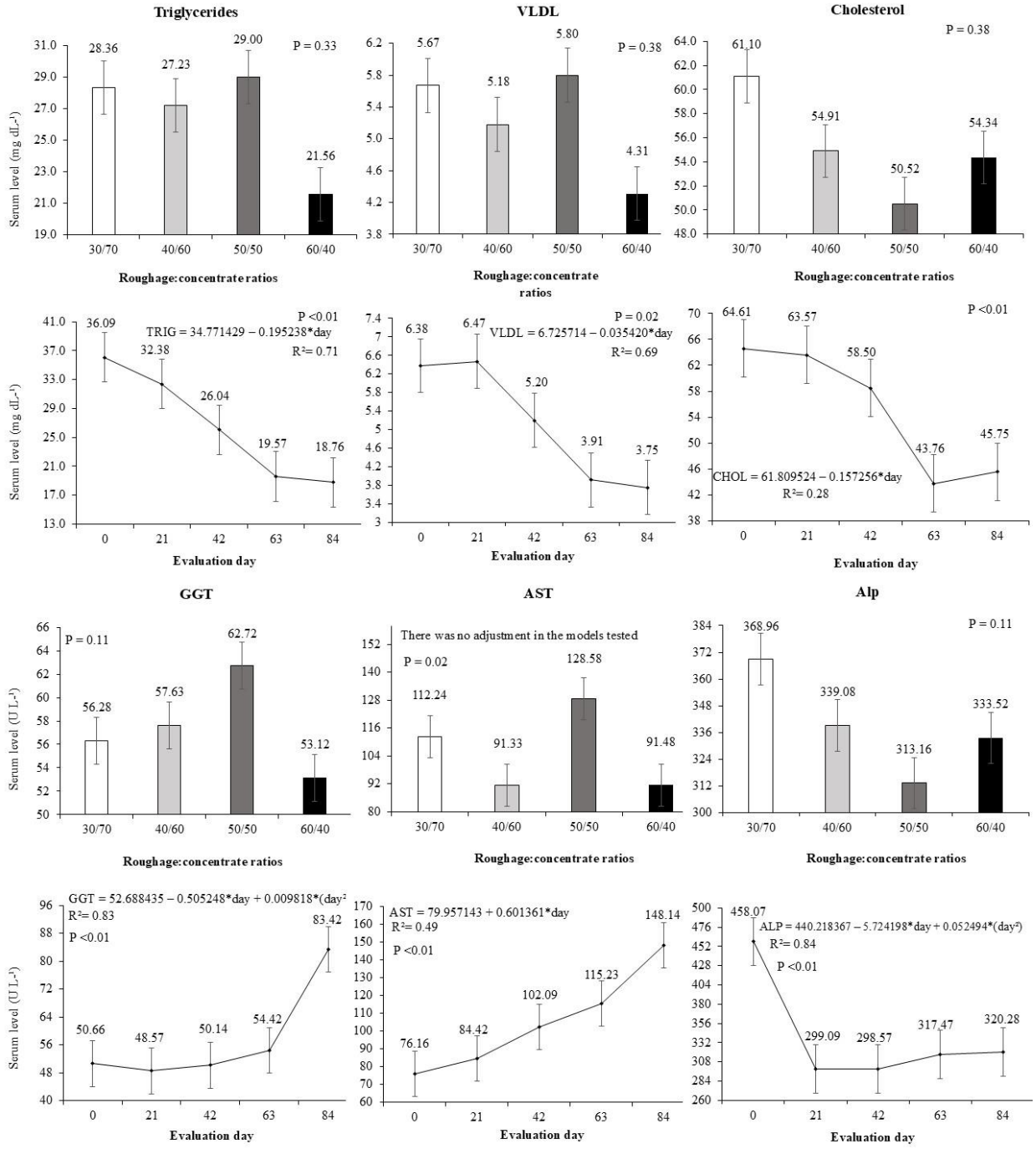

Figure 3. Energy and enzyme profile of lambs fed extruded diets in different roughage: concentrate ratios and in different periods.

Discussion

Effect of roughage:concentrate ratio on performance, feeding behavior and metabolism of sheep fed extruded diet

Diets rich in roughage physically limit nutrient intake due to the physical filling of the rumen by the fiber, with an increase in time and energy spent on ingestive activities and a reduction in productive weight gain (Parente et al., 2016; Faryabi et al., 2023; Oliveira et al., 2024). Oliveira et al. (2025) found no effect of R:C in fully extruded diets on intake (1.51 kg/day) and dry matter digestibility (67.03%) in sheep confined in individual facilities. Our study was carried out in collective pens, limiting the measurement of nutrient intake per animal. However, our findings weight gain performance and feeding behavior corroborate those of Oliveira et al. (2025), allowing us to infer similar nutrient intake per animal in our study.

The similarity of time in rumination with an increase in roughage in the diet describes unusual behavior in ruminant nutrition. Decomposition of plant cells and partial breakdown of fibrous structures are promoted by extrusion (Gjorgjievski et al., 2022; Brand et al., 2023; Ghorbani et al., 2020), altering the stability of the fiber and making it easier and faster to be degraded. This improves the rates of nutrient and energy availability in roughage, and results in similarity in metabolism, feeding behavior, and weight gain performance of the animal on the various R:C diets.

Diet is one of the main factors affecting biochemical parameters (Varanis et al., 2021). Although the nutritional composition of the experimental diets changed with an increase in roughage, the serum metabolites were similar among all sheep, as also observed by Oliveira et al. (2025). The absence of differences among R:C diets may be an effect of the greater availability of nutrients for metabolization due to the extrusion of the roughage, allowing all animals to reach a plateau in nutrient assimilation rates in the body. The similarity in urea and uric acid levels among the different R:C diets suggest similarity in the availability and metabolism of protein in the rumen, ruminal metabolism rate, and number of microorganisms present in the rumen (Varanis et al., 2021). The urea circulating in the blood reflects the ammonia synthesis profile in the rumen (Aliyu et al., 2012) and is used as non-protein nitrogen by microorganisms for the synthesis of microbial protein. The urea is degraded by bacteria and ruminal protozoa in the abomasum and intestine, which provides amino acids to the liver where they are incorporated into animal proteins (Bacila, 2003; Varanis et al., 2021) and later to body tissues, resulting in the absence of an effect on body weight gain performance.

The presence of albumin, synthesized from cysteine resulting from the degradation of glutathione by the action of GGT (Borburema et al., 2012; Varanis et al., 2021), indicates the availability of protein in the body, especially when associated with GGT levels. Creatinine is an indicator of the process of muscle growth (Varanis et al., 2021). Evaluating the effect of diet supplementation in early weaned lambs, Fernandes et al. (2012) observed that the reduction of creatinine in non-supplemented lambs resulted in a reduction in the renewal rates of muscle energy reserves in response to the low energy density of the ingested forage. Thus, the homogeneity of albumin,

creatinine, and GGT levels among the four R:C diets in our study confirms that, even with the reduction of protein in the diet, the protein and energy intake into the body – as a possible response to the Pmic rate described above – was not compromised, resulting in similar ADG among the diets during the study period (Table 2).

For growing animals consuming low energy nutritional food, the body uses triglycerides as a source of energy substrate for deposition in the muscles, which is directly associated with a reduction in the blood concentration of this metabolite (Santos et al., 2015; Varanis et al., 2021). In our study, the similarities in the concentration of triglycerides, VLDL - a lipoprotein that transports triglycerides for adipose deposition – and cholesterol – a constituent of lipoproteins that are synthesized in the liver and small intestine and act in the transport of lipids in the body (Kaneko et al., 2008; Santos et al., 2015) - indicate similarities in the rate of mobilization of lipids to the tissues and availability of energy for the animals in all the R:C treatments, as manifested by weight gain performance and feeding behavior. Although the energy metabolite levels were similar, the higher AST levels observed with diets with 30 and 50% roughage may indicate a greater need for energy mobilization to achieve the similarity described for energy metabolism in sheep. Evaluating high concentrate diets (90%) for sheep, Gregório et al. (2024) found results equivalent to 106.5 per UL of AST, close to the highest concentrations in our study. However, Gregório et al. (2024) found no liver injury during post-slaughter liver evaluation, corroborating our observations of VLDL, cholesterol, and triglycerides. This indicates that there was possible increased metabolism of energy compounds in the liver in response to greater energy availability but not enough to compromise the liver health of the animals.

Although there was no change in the concentration of energy metabolites in relation to R:C diet, it is noteworthy that the levels of roughage producing higher AST also induced an increase in the concentration of total proteins over time. This may indicate that these diets increased the availability of protein in the body and created a greater amount of lipids as an energy source, thereby optimizing the synergy of degradation of these nutrients, especially for muscle growth. This finding highlights the need for more studies that describe the integration of lipid/energy and protein metabolism with extruded diets.

Effect of time on metabolic parameters of sheep fed extruded diet in different ratios of roughage:concentrate

Creatinine is significantly affected by increased muscle mass as the animal ages (Varanis et al., 2021; Teixeira et al., 2021). In a performance study of sheep fed the same diets as those in our study, Macedo et al. (2019) described a reduction in weight gain in the period d30–d45, close to the creatinine reduction on d39 in our study. This proximity in time for weight gain and decreased creatinine corroborates the findings of Fernandes et al. (2012) who found that lower creatinine levels indicate lower intensity of protein metabolism associated with muscle tissue synthesis and deposition. The linear increase over time of albumin and total protein concentrations with 30 and 50% of roughage may be understood

as a reduction in protein deposition in tissues and an increase in the concentration of these metabolites in the blood, especially during d30–d45.

Taken altogether, we may infer that the possible reduction in the rate of muscle protein deposition occurred as a limitation of dietary energy over time, since the concentrations of triglycerides and VLDL reduced linearly during the 90-day sampling period, indicating the use of these metabolites as an energy substrate for muscle growth in the sheep. As a result, the increase in lipid mobilization over time increased AST and Alp levels, a behavior associated with an increase in the mobilization of energy compounds in the body, especially when associated with a reduction in cholesterol and albumin, as observed in this study. However, AST and Alp levels remained within the range described by Varanis et al. (2021), indicating the absence of hepatocellular lesions and no adverse effect on liver health.

Although the sheep in all diet treatments used triglycerides as energy, it is possible that this change in substrate physiologically influenced the use of energy and protein available for protein and muscle deposition in the body. The result was a reduction in the efficiency of amino acid use and protein synthesis around d26, manifested as a reduction in the level of GGT, accompanied by a linear increase in albumin and total serum proteins, while changing the ADG over time (Macedo et al., 2019). More studies focused on metabolism need to be developed to elucidate the correlations between lipid and protein metabolic pathways, especially in fully extruded diets.

Conclusion

Fully extruded diets with 30 and 50% roughage elevate protein and liver metabolism through total proteins and AST in the blood, but without compromising the health of the animals. Up to 60% roughage in fully extruded diets does not compromise the weight gain and feeding behavior of growing sheep, making it possible to increase the efficiency of fiber use in these ruminants.

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