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ANIMAL PRODUCTION

Megathyrsus maximus addition in total mixed ration on performance, metabolism, and quality of lamb carcasses

Milton Augusto Cassiano Sant'Anna[1] **Matheus Sousa de Paula Carlis**[2] **Daniel Montanher Polizel**[3] **Evandro Maia Ferreira**[2] **Cristiana Andrighetto**[4] **Patrícia Aparecida da Luz**[4] **Leandro Coelho de Araujo**[1*]

[1]Departamento de Biologia e Zootecnia, Faculdade de Engenharia do Campus de Ilha Solteira, Universidade Estadual Paulista "Júlio de Mesquita Filho" (UNESP), 15385-000, Ilha Solteira, SP, Brasil. E-mail: leandro.araujo@unesp.br. [*]Corresponding author.
[2]Departamento de Zootecnia, Escola Superior de Agricultura "Luiz de Queiroz", Universidade de São Paulo (USP), Piracicaba, SP, Brasil.
[3]Departamento de Nutrição e Produção Animal, Faculdade de Medicina Veterinária e Zootecnia, Universidade de São Paulo (USP), Pirassununga, SP, Brasil.
[4]Departamento de Produção Animal, Faculdade de Ciências Agrárias e Tecnológicas, Universidade Estadual Paulista "Júlio de Mesquita Filho" (UNESP), Dracena, SP, Brasil.

ABSTRACT: This study assessed the impact of total mixed ration silage of *Megathyrsus maximus* harvested with 70 cm composed of *Megathyrsus maximus* harvested at 70 cm in height and concentrate at the time of silage or total mixed ration of grass cut at 70 cm composed of *Megathyrsus maximus* harvested with 70 cm in height (in Exp. I and III) and total mixed ration silage cut at 130 cm composed of *Megathyrsus maximus* harvested 130 cm height and concentrate at the time of silage or total mixed ration of *Megathyrsus maximus* harvested with 130 cm composed of *Megathyrsus maximus* harvested at 130 cm height (in Exp. II and IV), on rumen metabolism, nutrient digestibility, and the performance and carcass characteristics of feedlot lambs. The results showed that TMRS70 increased ruminal pH in Exp. I and TMRS130 exhibited higher ether extract digestibility, propionic concentration, and lower acetic to propionic ratio and ruminal pH compared to TMR130 in Exp. II. In Exp. III and IV, there was no significant effect on the performance and quality of the carcass. However, in Exp. IV, TMRS130 led to decreased dry matter intake and average daily gain, while lambs fed with TMR130 showed a higher rib-eye area. Total ration ensilage could be used as a conservation method for young grasses (70 cm) without impairing performance, metabolism, carcasses, and meat quality. However, further research is recommended to evaluate the economic analysis of feeding total ration ensilage (70 cm) instead of total mixed ration as a forage conservation method.
Key words: feed conversion efficiency, lambs, tropical pastures, animal nutrition.

Efeito da adição de *Megathyrsus maximus* em ração total mista sobre desempenho, metabolismo e qualidade de carcaças de cordeiros

RESUMO: Este estudo teve como objetivo avaliar o impacto da silagem de ração total de *Megathyrsus maximus* colhido com 70 cm de altura e concentrado no momento da silagem ou ração total de capim cortado a 70 cm, composta por *Megathyrsus maximus* colhido com 70 cm de altura (nos Exp. I e III) e silagem de ração total cortada a 130 cm, composta por *Megathyrsus maximus* colhido com 130 cm de altura e concentrado no momento da silagem ou ração total de *Megathyrsus maximus* colhido a 130 cm de altura (nos Exp. II e IV), sobre o metabolismo ruminal, a digestibilidade dos nutrientes, e o desempenho e as características de carcaça de cordeiros confinados. Os resultados mostraram que TMRS70 aumentou o pH ruminal no Exp. I, e TMRS130 exibiu maior digestibilidade do extrato etéreo, concentração de propionato e menor razão acético/propionato e pH ruminal em comparação com TMR130 no Exp. II. Nos Exp. III e IV, não houve efeito significativo sobre o desempenho e a qualidade da carcaça. No entanto, no Exp. IV, TMRS130 levou a uma diminuição no consumo de matéria seca e no ganho médio diário, enquanto cordeiros alimentados com TMR130 mostraram uma maior área de olho de lombo. A ensilagem da ração total poderia ser usada como um método de conservação para gramíneas jovens (70 cm) sem prejudicar o desempenho, metabolismo, carcaças e qualidade da carne. No entanto, recomenda-se mais estudos avaliando a análise econômica da alimentação com ensilagem de ração total (70 cm) em vez de ração totalmente misturada como método de conservação de forragem.
Palavras-chave: eficiência alimentar, cordeiros, pastagens tropicais, nutrição animal.

INTRODUCTION

The conservation of forage in the season of greater production of green mass is a strategy used to guarantee productive gains throughout the year, guaranteeing the supply of feed in the dry season, when there is a shortage of forage production. Programming silage conservation from the abundant grass mass during the rainy season can reduce mass losses and grazing management issues, such as

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difficulty in achieving pre-grazing condition goals (DA SILVA et al., 2008). In this way, the ensilage of young tropical grass with high nutrient content can condition a better performance of the animals by providing greater dry matter intake (DMI), and nutrient digestibility. However, the tropical forage grasses present limitations in the contents of dry matter (DM) and soluble carbohydrates, which can affect the fermentation process, increase dry matter losses, and reduce the nutritional quality of the silage (OUDE ELFERINK et al., 1999). To improve forage characteristics favoring fermentation in the silo, the incorporation of concentrate can be a strategy to enhance the final quality of the fermented material, characterizing it as total mixed ration silage (TMRS).

The TMRS results from the silage process of the total mixed ration, with research evaluating this technique since the 60s (OWEN & HOWARD, 1965). The TMRS contains high concentrations of organic acids despite the high content of dry matter, which is generally between 40 to 600 g/kg (NISHINO et al., 2004; WANG & NISHINO, 2008; WEINBERG et al., 2011) and can be stored in silos in a similar way to the traditional roughage exclusive silage, saving time in the daily preparation of the herd's feed. Several studies have reported that total mixed ration silage is stable after opening, even at higher temperatures (NISHINO et al., 2004; WANG & NISHINO, 2008; WANG & NISHINHO, 2013), providing stability of the mixture even in hot climates (KONDO et al., 2015). In Asian countries, TMRS has been increasingly investigated, and experiments have been carried out with the objective of reusing co-products of higher moisture and nutritional value, such as those from mushroom cultivation, soy sauce, and breweries, by simultaneous silage with bulky and concentrated and the results have been promising. Despite the scarcity of experiments evaluating animal performance (SAKAI et al., 2015), positive effects were observed in the characteristics of the composition of the TMRS and the rumen fermentation profile (NISHINO et al., 2004; WANG & NISHINO, 2008; UDDIN et al., 2009; XU et al., 2010; XU et al., 2007; WEINBERG et al., 2011; KONDO et al., 2015). In tropical countries, adopting TMRS can be strategic in storing the forage produced in large quantities during the rainy season (DA SILVA et al., 2008).

Changes in the nutritional composition of ensiled TMR may occur due to the fermentation process, such as the increase in starch digestibility by proteolysis of the protein matrix during silage fermentation (HOFFMAN et al., 2011) and the increase in the soluble protein fraction and concentration of ammonia (LAZZARI et al., 2021). Conversely, TOMAZ (2018) evaluated the fermentation capacity of *Megathyrsus maximus* cut at different heights and reported that the lowest fermentation capacity was observed for the height of 70 cm, whereas the highest fermentation capacity was observed for grass cut at 130 cm. In this scenario, it is important to evaluate the interaction of the ingredients and grass maturity stage used to produce TMR silage to optimize the use of nutrients by the animals.

In the present study, it was proposed to compare the animal performance, rumen fermentation, and carcass quality of feedlot lambs fed total mixed ration silage of *Megathyrsus maximus* harvested with 70 cm composed of *Megathyrsus maximus* harvested at 70 cm in height and concentrate at the time of silage or total mixed ration of grass cut at 70 cm composed of *Megathyrsus maximus* harvested with 70 cm in height (in Exp. I and III) and total mixed ration silage cut at 130 cm composed of *Megathyrsus maximus* harvested 130 cm height and concentrate at the time of silage or total mixed ration of *Megathyrsus maximus* harvested with 130 cm composed of *Megathyrsus maximus* harvested at 130 cm height (in Exp. II and IV). The heights were previously proposed to evaluate the grass maturity stage, which changes the quality of the protein and fiber of the forage used in the preparation of the diets (TMR and TMRS).

MATERIALS AND METHODS

These studies were conducted at the Universidade de São Paulo, Piracicaba campus (USP/ESALQ; Piracicaba, SP, Brazil; 22°43'31"S, 47°38'51"W and 524m elevation; Exp. I and Exp. II) and at the Sao Paulo State University, Ilha Solteira Campus (UNESP; Ilha Solteira/SP, Brazil; 20º25'58"S, 51°20'33"W and 335 m elevation; Exp. III and IV). For all experiments, *Megathyrsus maximus* was harvested in the first rainy season (from March 2018 to April 2019), stored away from the sun and rain at the Farm for Teaching and Research and Extension of the Animal Production Sector and at Universidade Estadual Paulista, Campus Ilha Solteira, located in Selvíria -MS (21º22'S, 51º24'W and 348 m altitude) and used for the experiments in 2019. The harvest was carried out with a grass harvester platform coupled to a (JF C120 – Itapira – Brazil) silage harvester (0.20 m residue), with adjustment to obtain an average particle size of 2 cm. For use of the total mixed ration silage (total feed silage cut at 70 cm and total mixed ration silage cut at 130 cm), the chopped grass was mixed to the concentrate using a wagon for silage mixture (Siltomac, model S 4.3 – Saint Charles - Brazil) and later the mixture was compacted in plastic bags with a capacity of approximately 40 kg and suitable for use in forage filler with hydraulic system (model PS4000, Trapani – Black Stream - Brazil). For the height of 70 cm, it was necessary to lower the cutting height of the ensile to approximately 10 cm in height, since at a height greater than that, the implement was not able to dump the chopped material.

The grass silage with citrus pulp for the composition of the total mixed ration (total feed cut at 70 cm and total mixed ration cut at 130 cm) was ensiled with the same equipment but without adding the other ingredients (concentrate). All silages had a target density of approximately 493 ± 33 kg of green mass m3 and were inoculated with a commercial product containing *Lactobacillus plantarum* (2.6 × 1010 CFU g^{-1}) and *Pediococcus pentasaceus* (2.6 × 1010 colony forming unit g^{-1}), as recommended by the manufacturer.

The diets were proposed to evaluate the effects of TMR ensiling for two different grass maturity stages (Table 1). The diets used in the four experiments were as follows:

Exp. I and II: 1) total mixed ration silage of *Megathyrsus maximus* harvested with 70 cm (TMRS70) - composed of *Megathyrsus maximus* harvested at 70 cm in height and 500 g of

Table 1 - Where in each experiment, each animal received the two treatments in two different periods, totaling two repetitions for each treatment in each experiment.

Ingredients (g/kg of DM)	Diets[1]			
	Exp. I and III		Exp. II and IV	
	TMR70	TMRS70	TMR 130	TMRS130
Silage				
Megathyrsus maximus	500	500	500	500
Citrus Pulp	150	150	150	150
Soybean meal	—	30	—	188
Ground corn	—	300	—	142
Mineral mixture	—	20	—	20
Concentrate				
Citrus pulp	—	—	—	—
Soybean meal	30	—	188	—
Ground corn	300	—	142	—
Mineral mixture	20	—	20	—
Chemical composition				
Dry matter (g/kg as-fed basis)	293.2	259.5	350.5	294.6
Crude protein, g/kg of DM	126.3	136.9	216.4	209.7
Ether extract, g/kg of DM	29.4	31.4	22.8	26.7
Ash, g/kg of DM	106.3	105.3	94.9	98.1
Neutral detergent fiber, g/kg of DM[4]	555.7	552.7	479.6	478.6
Acid detergent fiber, g/kg of DM	279.6	274.4	224.1	247.0
Non-fibrous carbohydrates	182.3	173.7	186.3	186.9
Lignin, g/kg of DM	34.2	23.1	33.4	28.6
Cellulose, g/kg of DM	245.4	251.3	190.7	218.4
Nitrogen unavailable inneutral detergent, g/kg of DM	4.0	3.3	8.1	3.4
Nitrogen unavailable in acid detergent, g/kg of DM	1.8	1.8	2.1	2.6

[1]TMR70 = total mixed ration containing 50% of *Megathyrsus maximus* silage harvested with 70 cm of height; TMRS70 = total mixed ration silage containing 50% of *Megathyrsus maximus* harvested with 70 cm of height; TMR130 = total mixed ration containing 50% of *Megathyrsus maximus* silage harvested with 130 cm of height; TMRS130 = total mixed ration silage containing 50% of *Megathyrsus maximus* harvested with 130 cm of height.

concentrate per kg of DM at the time of silage; 2) total mixed ration of grass cut at 70 cm (TMR70) - composed of *Megathyrsus maximus* harvested with 70 cm in height and ensiled with 150 g of dry citrus pulp per kg of DM + 350 g of concentrate per kg of DM (daily mixed at the time of offering the experimental diets).

Exp. II and IV: 3) total mixed ration silage cut at 130 cm (TMRS130) - composed of *Megathyrsus maximus* harvested 130 cm height and 500 g of concentrate per kg of DM at the time of silage;4) total mixed ration of *Megathyrsus maximus* harvested with 130 cm (TMR130) - composed of *Megathyrsus maximus* harvested at 130 cm height ensiled with 150 g of dry citrus pulp per kg of DM+ 350 g of concentrate per kg of DM (daily mixed at the time of offering the experimental diets).

The experimental diets were formulated to be isonitrogenated (in each experiment), using the NRC (2007) recommendations. The diets predicted a minimum average weight gain of 214 g/day, using the average composition of *Megathyrsus maximus* grass at the respective heights (TOMAZ et al., 2018).

The animals were fed once a day at 8 am, the silos were opened for immediate use, and the remaining silage was discarded. The total mixed ration silage (TMRS70 and TMRS130) was placed directly in the feeders while the total mixed ration (TMR70 and TMR130) and their respective concentrate were weighed separately on a precision electronic scale of 1 g (Toledo, 9094 PLUS – Saint Bernard of the Field) after weighing the silage and the concentrate were mixed and offered to the animals for the treatments TMR70 and TMR130. All animals had ad libitum access to diets and drinking water. The following day, before weighing the experimental diets, the trough readings were performed to define the quantity to be offered, whose leftovers were kept below 100 g/kg of the quantity offered.

Experiments I and II: animal metabolism
Animals and experimental design

The total of the four ruminally cannulated Dorper × Santa Inês with initial body weight (BW) of 75.62 ± 4.58 kg (n = 2, Exp. I) and 75.82 ± 5.53 kg (n = 2, Exp. II), approximately 12 months old, were assigned to individual metabolism cages, with dimensions of 1.30 × 0.55 m provided with a feed bunk, waterer, and a system for feces and urine collection. The metabolism cages were kept indoors and protected from direct sunlight and rain. On the first day, the lambs were dewormed with 10g/kg moxidectin (Cydectin, Fort Dodge Saúde Animal, Campinas, São Paulo, Brazil) at 1 mL/50 kg of BW.

The withers were randomly distributed in a 2 × 2 crossover experimental design, with two treatments, two 21-day periods, and four repetitions in each experiment. The first 14 days of each period were for the adaptation of animals to experimental diets, and the last 7 days were for data collection.

Digestibility of nutrients and nitrogen balance

Between the 16th and 20th day of each period of experiments I and II, leftovers were recorded daily for DMI calculations. A sample was taken daily from offered feed and leftovers of each experimental unit, composed of animals, and kept at -18 °C for analysis. The total fecal and urine production was collected daily using collection bags to avoid contamination. Urine was collected in containers with 6 N HCl to prevent ammonia volatilization, keeping the pH below 3.0. Feces and urine were weighed at 08h00, and a sample (100g/kg of the total weight) of the daily production of each was collected and stored at -18 °C.

Total tract apparent nutrient digestibility was calculated according to the formula: TTAD (%) = ((DMI × NCDM) – (FDM × NCFM) × 100)/(DMI × NCDM), where TTAD = total tract apparent digestibility, DMI = dry matter intake, NCDM = nutrient content of the DMI (%), FDM = fecal dry matter, and NCFM = nutrient content of the fecal DM (%). The nitrogen balance was calculated according to the formulas that follow: N retention (g/day) = (Nintake - Nfeces - Nurine); N retention (%N intake) = [(Nintake - Nfeces - Nurine) /Nintake]; N retention (%N absorbed) = [(Nintake - Nfeces - Nurine) / (Nintake × N absorbed)].

Ruminal fermentation characteristics

Ruminal fluid samples were collected on the 21st day of each experimental period. Collections were performed at hour 0 (before the experimental diets were offered), 3, 6, 9, 12, 18, and 24 after the diet was provided. The ruminal content samples were manually collected via cannula and then filtered through nylon cloth, and the ruminal fluid pH was immediately measured (Digimed-M20; Digimed Instrumentação Analítica; São Paulo, SP, Brazil). The solid phase of the rumen content that remained in the tissue after filtration was returned to the rumen. After determining the pH, the aliquots of the ruminal fluid were removed and stored in microtubes

(Eppendorf, São Paulo, Brazil) at -18 °C for later determination of rumen ammonia and the molar proportion of short-chain fatty acid (SCFA; acetate, propionate, butyrate, isobutyrate and isovalerate), as well as the acetate to propionate ratio (Ac: Prop) and total SCFA concentration. The SCFA in rumen fluid was determined according to FERREIRA et al. (2016), and the rumen ammonia was determined by the colorimetric method described by CHANEY and MARBACH (1962), adapted for reading in a microplate reader (BIO-RAD, Hercules, CA, USA), using a 550 nm absorbance filter.

Experiments III and IV (UNESP Ilha Solteira)
Animals and experimental design

A total of thirty-two Dorper × Santa Ines non-castrated lambs, with an initial BW of 18.31 ± 2.07 kg (n = 16, Exp. III) and 18.35 ± 2.60 (n = 16, Exp. IV), were assigned to pens in a complete block design with two blocks (n=8 animals, two blocks in each trail) in each trail, the blocks were defined according to their shrunk BW (after 14 h of feed restriction). All animals were dewormed with 75 g/kg Levamisole Phosphate at the dosage of 1 ml/40 kg of BW and received application of ADE vitamin supplement on the first day of adaptation.

The experimental period lasted 56 days. The leftovers were recorded weekly to determine the DMI, and the feed amount was calculated according to previous intake, adjusting when needed to ensure that refusals did not exceed 50g/kg of daily intake. The lambs were weighed after fasting for 14h on days 0 and 56, and the ADG and feed efficiency (FE) were calculated.

Slaughter of animals, carcass characteristics and sampling

At the end of the experimental period of both experiments, all animals were slaughtered at Bertin Refrigerator, Lins-SP. The process was routine, following the norms described in the Regulation of the Industrial and Sanitary Inspection of Products of Animal Origin – RIISPOA (1980). After bleeding, skinning, and evisceration, the hot carcass weight (HCW) was recorded, and after 24 h of cooling at 4 °C, the cold carcass weight (CCW) was re-weighted. Hot carcass yield (HCY), cold carcass yield (CCY), and cooling loss (CL) were calculated using the formulas: hot carcass yield (hot carcass weight /animals' body weight) × 100; cold carcass yield (cold carcass yield/animals' body weight) x 100 and cooling loss [(hot carcass weight – cold carcass weight) / hot carcass weight)] x100, with slaughter body weight (SBW) being the animal's body weight at the moment before slaughter.

Backfat thickness (BFT) was measured on the Longissimus thoracis muscle between the 12th and 13th ribs. After 24 h of chilling, the L. thoracis muscle was sectioned in a transverse manner, and the subcutaneous fat thickness was determined on both sides of the carcass using a digital caliper (Motutoyo, Suzano, Brazil) graduated in millimeters. The exposed face of the L. thoracis muscle was drawn on parchment paper and subsequently scanned, and its area was measured with the aid of the ImageJ program to obtain the ribeye area (REA) according to SILVA SOBRINHO et al. (1999).

Laboratory analysis and calculations

Feed, orts, and feces samples were dried in a forced-air oven at 55 °C for 72 h. Then all samples were ground with a Wiley mill (Marconi, Piracicaba, São Paulo, Brazil) to pass a 1mm screen. Dry matter was determined by oven-drying at 105 °C for 24 h according to the method of the Association of Official Analytical Chemists (AOAC, 1990; #934.01), and OM was determined by incinerating the sample in a muffle furnace at 550 °C for 4 h (method 942.05; AOAC, 1990). The crude protein (CP), by total N determination, using the micro-Kjeldahl technique (method 920.87; AOAC, 1990) and the ether extract was determined with a high level of precision, using a rapid and efficient high-temperature solvent extraction method (method 996.06; AOAC, 2005). The NDF and ADF concentrations were meticulously determined according to VAN SOEST et al. (1991), using heat-stable alpha-amylase and sodium sulfite for the NDF analysis.

Statistical analysis

The data were analyzed using the MIXED Procedure (SAS Inst., Inc., Cary, NC). The animal within each period was considered the experimental unit for experiments I and II for all variables analyzed. All data were analyzed using Kenward-Roger approximation to determine the denominator for the test of fixed effects. Model statements for the variables analyzed as repeated measures over time contained the fixed effect of treatment, time, and treatment × time interactions. For nutrient intake and digestibility, the model statement contained the fixed effect of treatment. The animal and period were considered as a random effect. The covariance structure used was first-order autoregressive, which provides the smallest Akaike information criterion corrected.

Experiments III and IV data were evaluated using a model statement containing a fixed effect of treatment and a random effect of block. All results are reported as least square means. Significance was set at P ≤ 0.05, and results are reported according to the main effect if no treatment and hour interaction were observed.

RESULTS

Experiment I

The TMRS did not affect the DMI and nutrient intake compared to TMR (Table 2). However, lambs fed TMRS exhibited higher intake (P = 0.01) and digestibility (P = 0.03) of non-fibrous carbohydrates (NFC) than those fed TMR. In addition, there was no treatment effect for nutrient digestibility (P > 0.05) and nitrogen balance (P > 0.05, Table 3).

There was no treatment and hour interaction for rumen fermentation parameters (P > 0.05; Table 4). The treatments did not affect the molar proportion of SCFA, Ac:Prop ratio, total SCFA concentration, and rumen ammonia. However, whether fed TMRS70 had a higher rumen pH than TMR70 (P < 0.01). There was an hour effect for isobutyrate (P = 0.03) and rumen pH (P < 0.01; Table 4).

Experiment II

There was no effect of treatment for DMI and nutrient intake (P > 0.05). The TMRS increased the ether extract digestibility (P = 0.01). No treatment effect was detected for DM, OM, CP, NDF, and ADF apparent nutrient digestibility (Table 2). In addition, there was no treatment effect for nitrogen balance (Table 3).

There was an interaction between treatment and collection time (Table 4) for the concentration of branched-chain fatty acids (isobutyric, P < 0.001 and isovaleric, P < 0.01), but only the variables of propionic, isovaleric, and ammoniacal nitrogen were not affected by the collection time. The total mixed ration showed a higher concentration of propionic (P < 0.0001), consequently a lower ratio of Ac: Prop (P < 0.001) and ruminal pH (P = 0.03) than TMRS.

Experiment III

There was no difference in the final body weight (FBW), ADG, DMI in kg/d, % to body

Table 2 - Intake and digestibility of nutrients in sheep fed with total mixed ration (TMR) or total mixed ration silage (TMRS) containing *Megathyrsus maximus* harvested at different grass maturity stages (70 or 130 cm).

Item[4]	------Experiment I[1]------		SEM	P-Value[3]	------Experiment II[2]------		SEM	P-Value[3]
	TMR70	TMRS70		T	TMR130	TMRS130		T
----------Intake, kg/d----------								
Dry matter	0.871	1.062	0.14	0.39	0.956	1.017	0.09	0.66
Organic matter	0.759	0.959	0.12	0.33	0.858	0.914	0.08	0.65
Crude protein	0.118	0.139	0.02	0.44	0.178	0.197	0.02	0.44
Ether extract	0.035	0.049	0.01	0.15	0.033	0.040	0.01	0.22
Neutral detergent fiber	0.488	0.419	0.07	0.53	0.554	0.526	0.05	0.69
Acid detergent fiber	0.284	0.220	0.04	0.37	0.325	0.297	0.03	0.52
Non-fibrous carbohydrates	0.117	0.343	0.03	0.01	0.095	0.151	0.02	0.06
----------Digestibility, g/kg of DM----------								
Dry matter	621.4	652.0	70.2	0.78	705.9	661.3	25.1	0.25
Organic matter	649.1	678.3	64.1	0.77	724.7	683.5	25.5	0.29
Crude protein	594.1	615.6	72.8	0.85	759.6	751.6	14.9	0.58
Ether extract	775.7	843.3	34.7	0.26	831.9	939.3	16.6	0.01
Neutral detergent fiber	652.9	660.2	68.6	0.94	725.0	670.6	29.7	0.28
Acid detergent fiber	585.9	557.5	76.9	0.83	679.8	594.2	38.2	0.18
Non-fibrous carbohydrates	651.7	835.1	32.6	0.03	618.1	572.2	4.36	0.37

[1]TMR70 = total mixed ration containing 50% of *Megathyrsus maximus* silage harvested with 70 cm of height; TMRS70 = total mixed ration silage containing 50% of *Megathyrsus maximus* harvested with 70 cm of height.
[2]TMR130 = total mixed ration containing 50% of *Megathyrsus maximus* silage harvested with 130 cm of height; TMRS130 = total mixed ration silage containing 50% of *Megathyrsus maximus* harvested with 130 cm of height.
[3]T = Treatments effects.

Table 3 - Nitrogen balance in sheep fed with total mixed ration (TMR) or total mixed ration silage (TMRS) containing *Megathyrsus maximus* harvested at different grass maturity stages (70 or 130 cm).

Item[4]	Experiment I[1]		SEM	P-Value[3]	-----------Experiment II[2]----------		SEM	P-Value[3]
	TMR70	TMRS70		T	TMR130	TMRS130		T
Nitrogen, g/day								
Intake	18.86	22.23	2.67	0.44	28.03	31.43	2.72	0.44
Fecal	7.36	8.12	1.46	0.71	6.74	7.89	0.90	0.43
Urinary	11.50	14.12	2.92	0.57	21.30	23.53	1.94	0.48
Absorbed	5.67	4.68	0.90	0.13	6.80	6.33	0.92	0.74
N Retention								
g/day	5.83	94.4	30.9	0.47	14.50	17.20	1.92	0.39
g/kg N intake	272.4	401.5	112.1	0.47	515.0	545.3	35.8	0.59
g/kg N absorbed	435.2	599.0	134.2	0.45	675.9	726.5	43.0	0.47

[1]TMR70 = total mixed ration containing 50% of *Megathyrsus maximus* silage harvested with 70 cm of height; TMRS70 = total mixed ration silage containing 50% of *Megathyrsus maximus* harvested with 70 cm of height.

[2]TMR130 = total mixed ration containing 50% of *Megathyrsus maximus* silage harvested with 130 cm of height; TMRS130 = total mixed ration silage containing 50% of *Megathyrsus maximus* harvested with 130 cm of height.

[3]T = treatment effect.

[4]g/kg N intake = percentage of nitrogen retained in relation to intake; g/kg of absorbed = percentage of nitrogen retained in relation to absorbed.

weight, g/kg of dry matter 0.75, and feed efficiency (FE, Table 5).

Experiment IV

Total mixed ration silage with grass harvested at 130 cm decreased DMI (P = 0.02), and ADG (P = 0.02). There was no difference for the other variables evaluated (P < 0.05, Table 5)

Regarding carcass and meat quality, lambs fed with TMR130 presented greater REA (P = 0.04) than lambs fed with the TMRS130 (Table 6).

DISCUSSION

Experiments I e II

In the present study, the composition of the experimental diets was very similar, which resulted in the absence of an effect on nutrient intake and digestibility when the TMR was ensiled for approximately one year. Total mixed ratio ensilage is capable of modifying protein fractionation and nutrient availability, with an increase in the proportion of soluble protein being reported (LAZZARI et al., 2021), an increase in starch digestibility (BUENO et al., 2020), and a decrease in the total carbohydrate content nevertheless no affect the NFC content (DU et al., 2020). Increases in digestibility are associated with changes in the bioavailability of nutrients present in the feed during the ensilage fermentation process, with an increase in digestibility being described in the literature when using TMRS (MIYAJI & NONAKA, 2018; MEENONGYAI et al., 2017).

In this sense, during the ensiling process, the effects on the fiber, mainly hemicellulose and cellulose, with little influence on the digestibility of the fiber fraction. The effects of ensiling on NDF loss during ensiling appear to depend on the ensiled material's DM value (WEINBERG et al., 2011). Furthermore, cell wall hydrolysis and changes in the fibrous fraction during ensiling depend more on the ingredients added to TMR (BUENO et al., 2020). The data obtained in the present study demonstrated that the ensiling of TMR with grass cut at 70 cm (Exp I) or 130 cm (Exp II) did not affect the digestibility of NDF or ADF.

However, the inclusion of concentrate during the preparation of TMRS70 may have provided a greater amount of water-soluble carbohydrates for the fermentation process, thereby decreasing the content of total carbohydrates, sugars, and soluble fiber, but without altering the NFC content at the end of the fermentation process (DU et al., 2020). In our study, there were no differences in NFC contents when comparing TMR and TMRS. However, due to the numerically higher DMI, lambs fed with TMRS70 exhibited greater intake and digestibility of NFC than TMR70.

Table 4 - Parameters of ruminal fermentation in sheep fed with total mixed ration (TMR) or total mixed ration silage (TMRS) containing *Megathyrsus maximus* harvested at different grass maturity stages (70 or 130 cm).

Item[4]	-----Experiment I[1]-----		SEM	-----------P-Value[3]---------			------Experiment II[2]------		SEM	-------------P-Value[3]-------------		
	TMR70	TMRS70		T	H	T×H	TMR130	TMRS130		T	H	T×H
Short-chain fatty acids –SCFA, mM												
Acetate	70.28	71.05	1.33	0.62	0.79	0.69	67.57	69.55	1.22	0.08	<0.01	0.06
Propionate	15.87	16.16	1.25	0.81	0.72	0.77	17.44	14.15	0.63	<0.0001	0.39	0.34
Isobutyrate	0.98	1.00	0.15	0.93	0.03	0.26	1.12	1.22	0.16	0.67	0.04	<0.001
Butyrate	9.84	9.40	1.00	0.75	0.51	0.13	10.96	12.03	0.67	0.29	0.05	0.06
Isovalerate	1.64	1.27	0.18	0.13	0.14	0.06	1.83	1.65	0.22	0.53	0.20	<0.01
Valerate	1.02	1.11	0.07	0.35	0.05	0.94	1.14	1.22	0.09	0.52	<0.001	0.10
Ac:Prop[4]	4.59	4.48	0.37	0.78	0.70	0.85	3.91	4.89	0.26	<0.001	<0.01	0.18
Total	96.67	84.03	14.25	0.21	0.07	0.64	94.41	92.85	5.32	0.83	<0.0001	0.96
pH	6.26	6.70	0.10	<0.01	<0.001	0.52	6.49	6.73	0.13	0.03	<0.0001	0.49
Ammonia, mg/dL	19.78	17.35	1.79	0.35	0.35	0.29	17.56	17.20	1.71	0.86	0.74	0.19

[1]TMR70 = total mixed ration containing 50% of *Megathyrsus maximus* silage harvested with 70 cm of height; TMRS70 = total mixed ration silage containing 50% of *Megathyrsus maximus* harvested with 70 cm of height.

[2]TMR130 = total mixed ration containing 50% of *Megathyrsus maximus* silage harvested with 130 cm of height; TMRS130 = total mixed ration silage containing 50% of *Megathyrsus maximus* harvested with 130 cm of height.

[3]T = treatment effect; H = effect of collection time; T×H = interaction between treatment and collection time.

[4]Ac: Prop = relationship between acetate and propionate.

In experiments I and II, the treatment effect for the ruminal pH can be explained due to the concentrate being mixed with silage at the time of supplying the diet and ensiled in the TMRS diets, thus being made available for fermentation in the total mixed ration silage conservation process. However, the addition of concentrate ingredients during the preparation of TMRS may have increased the concentration of SFA and decreased the pH of the TMRS. Nonetheless, it is recognized that there is no relationship between the pH of the consumed silage and the ruminal pH (ROOKE 1995, CHARMLEY, 2001), which is consistent with the present study.

Conversely, the TMR diets are made available as a substrate for microbial fermentation in the rumen, which gives a more significant fermentation peak in the first 3 hours after the intake and, consequently, a drop in rumen pH (NASEM, 2016). Since the starch and soluble carbohydrates in the concentrate have a high degradation rate, 2500 – 5000 g/kg/h (NASEM, 2016). With the supply of the concentrate at the time of supplying the TMR diets, there was a greater ruminal fermentation (KRAUSE & OETZEL, 2006), which suffered the effect of fermentation of non-structural carbohydrates due to the ensiling silage process of the forage and its concentrate.

The interaction between treatment and time for isobutyrate and isovalerate may be due to the regular oscillation of ruminal fermentation and pH during the day and the higher cellulose concentration in the TMRS130. This is due to the temporary alteration in the cellulolytic bacteria population and, consequently, in isobutyrate production. According to MARAIS et al. (1988), there is a direct relationship between the concentration of branched-chain fatty acids and cellulolytic bacteria, these fatty acids being essential for their growth.

Conversely, the TMRS130 presented a lower concentration of nitrogen unavailable in neutral detergent (Table 1), modifying the availability of CP for ruminal fermentation. In this regard, the lower concentration of rumen-degradable protein in TMR130 may have provided a lower concentration of branched-chain amino acids for the ruminal microbiota, consequently affecting isobutyrate production (WICKERSHAM et al., 2008), which can also be observed by the numerically higher nitrogen retention in lambs fed with TMRS130. In a study conducted by BATISTA et al. (2016), working with varying ruminal undegradable protein supplementation on forage digestion, they showed that the change in rumen-

Table 5 - Performance of lambs fed with total mixed ration (TMR) or total mixed ration silage (TMRS) containing *Megathyrsus maximus* harvested at different grass maturity stages (70 or 130 cm).

Item[4]	----------Exp. III [1]-----------			P-Value[3]	-----------Exp. IV [2]----------		SEM	P-Value[3]
	TMR70	TMRS70	SEM	T	TMR130	TMRS130		T
Initial BW, kg	20.85	21.26	0.77	0.43	21.03	21.15	0.79	0.68
Final BW, kg	29.28	28.98	1.14	0.84	34.60	30.83	1.40	0.07
Average daily gain, kg	0.151	0.138	0.02	0.53	0.242	0.173	0.02	0.02
----------Dry matter Intake----------								
kg/d	0.929	0.873	0.04	0.16	1.065	0.894	0.05	0.03
% to body weight	3.481	3.193	0.08	0.16	3.453	3.305	0.11	0.39
g/ kg de Dry matter^{0,75}	79.743	73.451	1.8	0.18	78.32	46.829	2.4	0.68
Feed Efficiency, ADG/DMI	0.163	0.156	0.01	0.73	0.228	0.189	0.01	0.07

[1]TMR70 = total mixed ration containing 50% of *Megathyrsus maximus* silage harvested with 70 cm of height; TMRS70 = total mixed ration silage containing 50% of *Megathyrsus maximus* harvested with 70 cm of height.
[2]TMR130 = total mixed ration containing 50% of *Megathyrsus maximus* silage harvested with 130 cm of height; TMRS130 = total mixed ration silage containing 50% of *Megathyrsus maximus* harvested with 130 cm of height.
[3]T = treatment effect.
[4]DMI = dry matter intake; ADG = average daily gain.

degradable protein could affect the availability of branched-chain amino acids and, consequently, the production of branched-chain fatty acids in the rumen. They also highlighted that there might be an interaction between these amino acids' availability and the cellulolytic bacteria population.

In experiment II, the higher propionate production, lower Ac: Prop ratio, and lower rumen pH can be explained in part by the numerically higher NFC digestibility de NFC in the TMR130 diet compared to the TMRS130 diet (Table 2), which contributes to the increasing the concentration of propionic acid. The starch is the main component of the NFC (non-fiber carbohydrates) of corn grain, which when ensiled (stored in silos) leads to an increase in its digestibility due to the degradation of the protein matrix that surrounds the starch granules present in the ground corn grain (FERRARETTO et al., 2013; HOFFMAN et al., 2011; GUSMÃO et al., 2018), thus increasing the concentration of propionic

Table 6 - Carcass and meat characteristics of lambs fed with total mixed ration (TMR) or total mixed ration silage (TMRS) containing *Megathyrsus maximus* harvested at different grass maturity stages (70 or 130 cm).

Item[4]	Experiment III[1]			P-Value[3]	Experiment IV[2]			P-Value[3]
	TMR70	TMRS70	SEM	T	TMR130	TMRS130	SEM	T
Hot carcass yield, g/kg	409.7	397.0	30.2	0.77	472.2	433.7	19.4	0.12
Cold carcass yield, g/kg	393.6	383.0	29.1	0.80	457.7	419.5	20.3	0.13
Subcutaneous fat thickness, mm	2.75	2.55	0.43	0.71	2.89	2.65	0.47	0.71
Body wall thickness, mm	14.20	14.17	0.90	0.98	16.38	14.03	0.92	0.09
Rib eye area, cm²	10.23	10.29	0.47	0.93	14.01	11.51	0.82	0.04

[1]TMR70 = total mixed ration containing 50% of *Megathyrsus maximus* silage harvested with 70 cm of height; TMRS70 = total mixed ration silage containing 50% of *Megathyrsus maximus* harvested with 70 cm of height.
[2]TMR130 = total mixed ration containing 50% of *Megathyrsus maximus* silage harvested with 130 cm of height; TMRS130 = total mixed ration silage containing 50% of *Megathyrsus maximus* harvested with 130 cm of height.
[3]T = treatment effect.

in the ruminal fluid (ØRSKOV, 1986) however these effects no founded in this work.

The inclusion of concentrate rich in starch during the preparation of TMRS130 may have been mostly consumed during the silage fermentation process due to the traditionally low water-soluble carbohydrate content in tropical grass silages (DU et al., 2020). In this context, we hypothesize that the numerically higher NFC digestibility and propionate concentration observed in lambs fed with TMR130 were due to starch's greater availability for ruminal fermentation than lambs fed with TMRS130.In the same sense, the lactate produced in the propionic route has a lower absorption rate by the ruminal epithelium and a lower pka (OETZEL, 2007), thus causing a decrease in the ruminal pH in the animals fed with the TMR130, however in both experiments (I and II) the ruminal pH remained in the recommended range of 5.5 to 6.5 (NAGARAJA & TITGEMEYER, 2007).

The present study observed increased EE digestibility for TMRS when the grass was harvested at 130 cm (Exp. II). This may be related to the higher concentration of EE in TMRS compared to TMR, which can be explained by the presence of concentrate ingredients that provide greater availability of compounds with oils, waxes, and pigments for microbial degradation. In this sense, GUSMÃO et al. (2018), working with TMRS containing Elephant grass, identified the lowest concentration of EE in the exclusive Elephant grass silage (1.67% DM) and observed positive increments in all treatments with the presence of concentrate ingredients in the composition, with the maximum value seen in SRT containing corn, soybean meal, and molasses (2.84% DM).

In that regard, LAZZARI et al. (2021) reported that increasing the amount of unsaturated lipids with the addition of soybean grain can improve the energy density of TMRS, increasing animal performance. However, the study did not evaluate the digestibility of the lipid fraction. We hypothesized that this increase in digestibility may be related to the growth of microorganisms during the fermentation process, which mainly have proteins and lipids in their composition. However, specific studies are needed to evaluate this hypothesis.

Experiments III e IV

The higher DMI by animals fed with the TMR130 diet may have been influenced by the content of SFA, due to the lower acetate concentration in this diet (Table 4). Corroborates with other authors who found that when there is an increase in the concentration of acetic acid and a decrease in the DMI in sheep (BUCHANAN-SMITH, 1990; NKOSSI & MESSKE, 2010). In addition, studies report a reduction in DMI in silages containing high concentrations of ammonia nitrogen (CUSHNAHAN et al., 1995; HUHTANEN et al., 2002). LAZZARI et al. (2021) reported a tendency to reduce DMI when using urea to produce TMRS, which explain the increase in the concentration of ammoniacal nitrogen. In experiment IV, soybean meal was used to produce the TMR. However, a greater inclusion was made for adjustments in the final crude protein content of the diet, which may have resulted in a greater accumulation of ammoniacal nitrogen and have negatively affected the DMI.

The higher ADG observed in the TMR130 diet can be explained by the larger DMI, which causes a greater supply of substrates for microbial growth, energy, and protein intake, as recommended by the NRC (2007) in which the ADG is correlated positively with the concentration of propionate, inferring in greater contribution and energy efficiency, thus allowing greater net gain energy for animals fed with these diets (RYLE & ØRSKOV, 1990). Such results corroborated those of MEENONGYAI et al. (2017), who obtained higher ADG in crossbred steers fed with TMR and related this effect to the higher propionate concentration.

Few studies have evaluated the effect of TMR and TMRS on carcass characteristics. When effects on these parameters are reported, the reasons are related to the composition of the evaluated diets. LAZZARI et al. (2021) reported that different nitrogen sources used for TMRS production did not affect carcass characteristics. However, they reported that when grain soybeans were used, there was an increase in hot carcass weight and Biceps femoris fat thickness, which the increased animal performance would explain due to the higher energy concentration of the diets and higher lipid content. In Exp III, the absence of treatment effect on carcass parameters can be justified by the performance results, in which the animal's final weight after the feedlot period was the same between treatments. Conversely, in experiment IV, lambs fed TMR130 showed higher average daily gain, and there was a tendency towards final weight gain. Animals with higher slaughter weight have greater muscle and fat deposition in the carcass (SILVA SOBRINHO et al., 1999). In the present study, the animals in the TMR130 treatment had an increase in the rib eye area and a tendency to increase in body wall thickness, variables that correspond to the increase in muscle and adipose tissue deposition, respectively.

CONCLUSION

Total ration ensilage could be used to conserve young grasses (70 cm) without impairing performance, metabolism, carcasses, and meat quality. However, it is not recommended for old grasses (130 cm), which may lead to reduced performance. Further research is recommended to evaluate the economic analysis of feeding total ration ensilage (70 cm) instead of total mixed ration as a forage conservation method.

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DECLARATION OF CONFLICT OF INTEREST

None of the authors declare to have a conflict of interest.

AUTHORS' CONTRIBUTIONS

MACS, EMF, MSPC and LCA conceived and designed experiments. MACS and MSPC performed the experiments, MACS, MSPC, CA and PAL carried out the lab analyses. LCA supervised and coordinated the project. DMP performed statistical analyses of experimental data. MACS, MSP, DMP and LCA prepared the draft of the manuscript. All authors critically revised the manuscript and approved of the final version.

BIOETHICS AND BIOSECURITY COMMITTEE APPROVAL

All animal procedures followed the guidelines recommended by the Ethics Committee for the Use of Animals CEUA (protocol No. 08/2016).

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