



# Dietary nutrient restrictions in the post-weaning period change Santa Inês ewe lamb nutritional metabolic profile

Clésio dos Santos Costa<sup>1</sup> · Marcos Cláudio Pinheiro Rogério<sup>2</sup> · Alexandre Lima Ferreira<sup>3</sup> ·  
Fernanda Samarini Machado<sup>4</sup> · Roberto Cláudio Fernandes Franco Pompeu<sup>2</sup> · Francisco Gleyson da Silveira Alves<sup>1</sup> ·  
João Paulo Arcelino do Rêgo<sup>5</sup> · Patrícia Guimarães Pimentel<sup>1</sup> · James Pierre Muir<sup>6</sup> · José Neuman Miranda Neiva<sup>7</sup>

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## Abstract

The objective of this study was to evaluate the metabolic profile of Santa Inês ewe lambs fed diets for early or late-maturing diets with or without nutrient restrictions. The experiment consisted of a  $2 \times 2$  completely randomized factorial experiment with either early- or late-maturity feed formulation according to “Nutrient Requirements of Small Ruminants” with or without 15% crude protein (CP) and total digestible nutrients (TDN) restrictions in diets formulated, five replications, and 20 ewe lambs averaging  $15.1 \pm 2.6$  kg. Lambs on early-maturity diets consumed greater ( $P < 0.05$ ) dietary ether extract (EE), non-fibrous carbohydrates, and TDN than those on late-maturity diets. Lambs on early-maturity diets had 7.11% greater dry matter digestibility (DMD) compared to lambs fed late-maturity diets. Lambs fed late-maturity diets, in general, had greater intake (IN), excreted (EN), and retained (RN) N as well as greater RN/IN and EN/IN ratios. There were no differences in blood total protein or albumin among lambs fed for different finishing maturity targets. Diets designed for late-maturing lambs resulted in greater microbial N and CP as well as rumen and metabolizable, degradable, and undegradable rumen and metabolizable CP. The selection of diets for early or late maturity carcasses depends on the production system goals. Diets without restrictions are recommended for early-maturity carcass finishing while diets with 15% CP and TDN restriction are recommended for late-maturity finishing.

**Keywords** Feed concentrate · Metabolizable energy · Sheep · Semiarid

## Introduction

The adaptive characteristics of local breeds to climatic conditions, low forage supply, include anatomical, physiological, hormonal, biochemical, and behavioral characteristics, all contributing to climate tolerance, diseases, and pasture feed (Bridi, 2001). However, seasonality of feed quantity and

nutritive value drives these production systems since there are periodic deficiencies that reduce efficiency (Pereira et al. 2018).

Production techniques, such as feed supplement that complements rangeland deficiencies or confined finishing, become important strategies under these conditions because they meet sheep nutritional needs. However, what nutrients local breeds

✉ Clésio dos Santos Costa  
clesiosantzoo@gmail.com

<sup>1</sup> Department of Animal Science, Federal University of Ceara, Avenida Mister Hull, 2977, Campus do Pici – Blocos 808-810, Postal Code 60, Fortaleza, Ceará .356-000, Brazil

<sup>2</sup> Brazilian Agricultural Research Corporation, Embrapa Goats & Sheep, Estrada Sobral-Groaíras, Km 04, Postal Code 62, Sobral, Ceará .011-970, Brazil

<sup>3</sup> Ministry of Agriculture, Livestock and Supply, Esplanada dos Ministérios - Bloco D – Brasília, Distrito Federal, Postal Code 70, Brasília, Federal District .043-900, Brazil

<sup>4</sup> Brazilian Agricultural Research Corporation, Embrapa Dairy Cattle, Rua Eugênio do Nascimento, 610 - Dom Bosco, Postal Code 36, Juiz de Fora, Minas Gerais .038-330, Brazil

<sup>5</sup> Federal Institute of Education, Science and Technology of Ceará, Postal Code 63, Boa Viagem, Ceará .870-000, Brazil

<sup>6</sup> Texas A&M AgriLife Research, 1229 North U.S. Hwy 281, Stephenville, TX 76401, USA

<sup>7</sup> Animal Science Department, Federal University of Tocantins, BR-153, Km 112, s/no, Caixa Postal 132, 77.804-970, Araguaina, Tocantins, Brazil

require under these harsh tropical semiarid conditions is unclear. The NRC (2007) offers two nutritional feed planes for feeding lambs: one for early finishing (diets high in energy) which encourage early fat deposition and another for late finishing which maximizes animal development by extending the growth curve when muscle deposition occurs and greater feed protein is required (Owens et al. 1993).

Research results on hair-sheep nutritional requirements in hot, dry tropical climates indicate that these differ from those reported by international committees because the latter depend on data derived from animal genetics and higher quality feeds from cooler climates (Pereira et al. 2017). As such, research on nutritional requirements of hair-sheep breeds from warm climates in hot conditions is needed to adjust the NRC (2007) recommendations to tropical climates (Galvani et al. 2008). Oliveira et al. (2020) for example, determined that reducing NRC (2007) dietary micronutrient feed requirements did not affect ram lamb feed consumption, digestibility nor animal performance. This reduction is in the order of 15% on average of the contents of protein and TDN in diets of sheep in native pasture (Araújo 2015; Carvalho 2019). Therefore, it is important to conduct studies with formulations in order to identify the adjustments in the requirements of animals raised in tropical environments (Oliveira et al. 2020). In working with animals of the Santa Inês breed, it was identified that the 15% adjustment did not change the growth curve of animals fed with diets of early maturity (Costa et al. 2020).

Our hypothesis was that a 15% dietary restriction of CP and TDN in diets formulated for early or late finishing would not affect Santa Inês ewe lamb feed intake or nutrient digestibility coefficients. Likewise, we hypothesize that these restrictions would improve N balance and optimize ruminal microbial synthesis without altering blood or ruminal metabolic profiles compared to diets without restrictions. The objective of this study was to evaluate the metabolic profile of Santa Inês ewe lambs fed diets for early or late-maturing diets with or without nutrient restrictions.

## Material and methods

### Animals, diets, and experimental design

All procedures were approved by the Empresa Brasileira de Pesquisa Agropecuária (Embrapa Caprinos e Ovinos, protocol n° 001/2017) Animal Use Ethics Committee. It was carried out at the Laboratório de Respirometria do Semiárido (LARESA) located in Sobral, Ceará, Brazil (3° 45' S, 40° 20' W, 110 m altitude). The climate is type BSh according to the Köppen classification. The experiment was designed as a completely randomized 2 × 2 factorial with early- or late-maturing finishing nutritional planes and 0 or 15% CP and TDN nutrition restriction according to NRC (2007)

recommendations for growing ewe lambs. All treatment combinations were replicated five times and each lamb was considered an experimental unit and fed individually. Twenty Santa Inês ewe lambs, all from the same October 2017 breeding season and average live weight (LW) of 15.09±2.63 kg and four month old, were confined for 110 days in metal metabolic crates. Diets were formulated for ewe lambs with 20 kg LW and 8 months of age to gain 200 g average daily gain (ADG). Ewe lambs were adapted to crates and diets for 14 days prior to initiating the trial. Feed was offered daily at 8:00 and 16:00 h. Feed-on-offer was constantly adjusted to allow up to 5% rejected orts, which were collected, weighed and subsampled daily.

### Sampling and laboratory analyses

For the test of feed consumption and digestibility, a collection period of 5 days was applied at the end of the experimental period. Feed samples were collected during that period in sufficient quantity for subsequent laboratory analyses. During that same period, rejected ort samples were collected from feeders immediately before morning feeding, weighed, subsampled, and stored. Total fecal and urine collections were obtained daily during this 5-day period using recipients under the metabolic cages. Feces were weighed, subsampled, and stored. Urine volume was measured, subsampled, and stored. Subsamples consisted of 20% of the total volume, per day and per animal, which were stored in plastic containers and stored in -10°C freezers until laboratory analyses were performed. At the end of the 5-day period, ort, fecal and urine samples were batched by animal.

Samples for urinary N were obtained via urine spot 3 h postprandially during 5 days using disposable colostomy bags (closed system, 30 mm, MEDSONDA®; Valadares Filho et al., 1999), fixed around the lamb vagina and adjusted for individual animal volume and weight. Of those samples, 20% was stored in plastic bottles with the addition of 10% chloric acid (HCl 2N) and stored in -10°C freezers until laboratory analysis.

At the end of the trial, feed, ort, and fecal samples were thawed and pre-desiccated in a forced-air oven set at 55°C until weight stabilized. These were then ground through a shear-mill fit with a 1-mm pore screen. The chemical analyses were carried out at the Animal Nutrition Laboratories of Embrapa Caprinos e Ovinos and the Universidade Federal do Ceará.

Dry matter (AOAC, 2005, 930.15), ash (AOAC, 2005, 942.05), CP (AOAC, 2005, 984.13), and EE (AOAC, 2005, 920.39) were assayed. All other laboratory procedures followed methods defined by the Brazilian National Institute of Science and Technology (INCT) as described by Detmann et al. (2012). Neutral detergent fiber (NDF; INCT-CA F-002/1), acid detergent fiber (ADF; INCT-CA F-004/1),

cellulose (CEL), hemicellulose (HCEL), and lignin (LIG; INCT-CA F-005/1) were determined. Protein insoluble in NDF (NDFN) was determined according to INCT-CA N-004/1 while protein insoluble in in ADF (ADFN) was assayed according to INCT-CA N-005/1. Correction for ash insoluble in NDF (NDFa) was determined according to INCT-CA M-002/1. Diet total digestible nutrients (TDN) were determined according to the equation proposed by NRC (2001). Total carbohydrate (TC) content were calculated according to Sniffen et al. (1992). Non-fibrous carbohydrates (NFC) were calculated according to Hall (2000).

A PARR 6100 bomb calorimeter was used to determine crude energy (CE) of feed, orts, feces and urine. These were carried out in the Universidade Federal do Ceará laboratories. Dry matter and nutrient intakes were calculated using the equation: Intake (g/d) = g nutrient fed – g nutrient rejected (orts). The equation proposed by Silva and Leão (1979) was used to calculate nutrient digestibility coefficients (DC): DC (%) = kg nutrient ingested – kg nutrients excreted in feces/kg nutrients ingested × 100. To determine N balance, urine samples were thawed and submitted to urine-N analysis by the semi-micro-Kjeldahl procedure (method INCT-CA N-001/1). Nitrogen intake (IN), fecal N (FN) and urinary N (UN) were assayed using the same procedure. The ratios FN/IN, UN/IN and retained N (RN) were based on these assays. Retained N was calculated according to Decandia et al. (2000):  $NR = NI - (NF + NU)$ .

Following data collection for feed intake and digestibility, undertaken in a single day, rumin liquid samples were collected. These were collected by inserting a silicone tube into lamb oral cavity as far as the rumin and using a vacuum pump (40-mm Hg pressure) to extract liquid. The liquid was filtered through a cotton cloth and pH was read using a digital potentiometer (Phmetro de bancada digital P1000, PHOX®). Subsamples of 50 mL were conditioned in 1-mL containers with 1:1 sulfuric acid and stored at –20°C until ammonial N (N-NH<sub>3</sub>) was determined. Ruminal N-NH<sub>3</sub> content was determined by distillation with magnesium oxide using boric acid as a color indicator (red from methyl bromide and green from bromocresol) titrated with HCl 0.01N.

Urine (voluntary urination) was collected using colostomy bags as described earlier. After collection, urine was filtered through three layers of gauze and 10 mL were added to 0.036 N sulfuric acid and stored at –20°C for later uric acid, allantoin, xanthine, and hypoxanthine quantification. Total purine (TP) excretion was estimated as the sum of urinary uric acid, allantoin, xanthine, and hypoxanthine. The quantity of microbial purines absorbed ( $X$  mmol/d) was estimated based on excreted purines ( $Y$ , mmol/d) (Chen and Gomes, 1992) for sheep using the equation  $Y = 0.84X + (0.150$

$LW^{0.75}e^{-0.25X}$ ), in which 0.84 is the factor of purine absorbed and  $0.150 LW^{0.75}$  is the endogenous contribution to purine excreted. Following Chen and Gomes (1992), microbial protein synthesized (MP) was calculated as  $MP$  (g/d) =  $X$  (mmol/d) ×  $70/0.116$  ×  $0.83$  ×  $1000$  =  $0.727 X$ , assuming 83% microbial purine digestibility, a 0.116 purine N:TN ratio and a purine N content of 70 mg N/mmol (Chen and Gomes, 1992).

To calculate degradable rumen protein (DRP), MP was multiplied by 1.11, corresponding to N recycling and ammonia loss (Marcondes et al., 2010). True digestible microbial protein synthesized (MPt) was calculated with the following equation:  $MPt = 0.64 \times DPR$ , where 0.64 is the correction factor based on 80% microbial protein digestibility and 80% intestinal digestibility (NRC, 2001).

Non-degradable rumen protein (NDRP) was calculated as CP ingested minus DRP. The NDRP digestibility (NDRPd) was calculated using the equation below assuming a fixed 80% NDRPd in the small intestine (NRC, 2001).  $NDRPd = NDRP \times 0.80$ . Metabolizable protein intake (MPI) was calculated by summing MPt and NDRPd (NRC, 2001) using the equation:  $MPI = MPt + NDRPd$ .

Blood metabolites were determined from lamb blood collected immediately prior to feeding by jugular venipuncture using 4.5-mL Vacutainer® tubes without anticoagulant. Samples were transported under refrigeration to the laboratory where they were centrifuged at 3500 rpm for 20 min. The supernatant plasma was placed in Eppendorf tubes and stored frozen at –10°C. Once thawed, these were assayed for TP, albumin, urea, creatine, glyucose, triglycerides, cholesterol and bilirubin content using a commercial kit (Labtest®, Lagoa Santa, MG, Brazil).

## Analyses of data

Data were analyzed for influencing values and outliers using residual studentized tests and all residual values exceeding ±2.5 were excluded tree values. The remaining data were submitted to normality test and adjusted as needed. They were then analyzed using PROC MIXED of the statistical program SAS® (Edition University, SAS Institute Inc., Cary, NC, USA; CODY 2015) using the following statistical model:

$$\text{Model 1: } Y_{ijk} = \mu + D_i + R_j + (D \times R_{ij}) + \varepsilon_{ijk},$$

where  $Y_{ijk}$  was the dependent variable collected during the trial, measured in ewe lambs of experimental unit “ $k$ ” of diet “ $i$ ” and diet restriction “ $j$ ”;  $\mu$  was the general constant;  $D_i$  the effect of diet “ $i$ ”;  $R_j$  the effect of nutrient restriction “ $j$ ”;  $D \times R_{ij}$  the effect of interaction between diets “ $i$ ” and nutrient restrictions “ $j$ ”; and  $\varepsilon_{ijk}$  the effect of random error. Means were obtained by command LSMEANS adjusted for Tukey’s test, considered different when  $P \leq 0.05$ .

**Table 1** Percentage and composition of diet ingredients

Composition % dry matter				
Dietary ingredients	E	E15	L	L15
Tifton Bermudagrass hay	16.92	38.55	37.93	62.63
Maize grain	66.67	55.79	33.47	15.19
Soybean meal	10.14	4.88	27.38	21.08
Vegetable oil	4.37	-	-	-
Calcium	0.89	0.78	1.22	1.10
Bicarbonate	1.00	-	-	-
Total	100	100	100	100
Chemical composition				
Dry matter <sup>a</sup>	94.20	93.90	94.00	93.90
Ash <sup>a</sup>	3.09	3.37	4.90	5.13
Ether extract <sup>b</sup>	6.96	2.62	2.21	2.04
Crude protein <sup>b</sup>	12.20	10.30	19.00	16.20
Neutral detergent fiber <sup>b</sup>	26.10	40.60	39.60	55.00
Acid detergent fiber <sup>b</sup>	9.60	16.90	17.60	25.70
Lignin <sup>b</sup>	2.24	3.64	3.49	5.01
Hemicellulose <sup>b</sup>	16.60	23.60	22.00	29.30
Cellulose <sup>b</sup>	9.10	17.40	18.00	27.30
Neutral detergent insoluble protein <sup>b</sup>	2.44	3.93	4.18	5.80
Acid detergent insoluble protein <sup>b</sup>	3.50	3.60	4.59	4.50
Total carbohydrate <sup>b,c</sup>	76.80	83.70	73.90	76.70
Non-fibrous carbohydrate <sup>b,d</sup>	53.10	47.10	38.60	27.50
Total digestible nutrients <sup>b,c</sup>	78.80	66.70	65.20	55.40
Ratio roughage:concentrate	17/83	38/62	38/62	62/38

E-Diet for early maturity without restrictions according to NRC (2007); E15-Diet for early maturity with 15% crude protein (CP) and total digestible nutrients (TDN) restricted according to NRC (2007); L-Diet for late maturity without restrictions according to NRC (2007); L15-Diets for late maturity with CP and TDN restricted by 15% according to NRC (2007); <sup>a</sup>Percentage of natural dry matter; <sup>b</sup>Percentage of dry matter; <sup>c</sup>Calculated according to Sniffen et al. (1992); <sup>d</sup>Calculated according to Weiss (1999); <sup>e</sup>Determined according to NRC (2001)

**Table 2** Santa Inês ewe lamb consumption of diets formulated according to NRC (2007) for early- and late-maturity with or without total digestible nutrient and crude protein restricted by 0 or 15%

	Maturity (M)		Restriction (R)		SE <sup>1</sup>	P-value		
	Early	Late	0%	15%		M	R	M*R
Consumption								
Dry matter	72.3	79.5	76.1	75.6	2.7	0.17	0.92	0.06
Organic matter	60.4	65.1	62.0	63.5	2.6	0.26	0.71	0.16
Crude protein	7.7B	13.0A	11.6a	9.2b	0.9	<0.001	<0.001	0.06
Ether extract	3.2A	1.6B	3.2a	1.6b	0.3	<0.001	<0.001	<0.001
Total carbohydrates	55.8	54.6	55.3	55.1	2.0	0.71	0.94	0.24
Non-fibrous carbohydrates	36.0A	25.4B	34.5a	26.9b	1.7	<0.001	0.001	0.06
Total digestible nutrients	50.6A	44.4B	52.3a	47.7b	2.3	0.04	0.006	0.24
Consumption as % body weight								
Neutral detergent fiber	1.0B	1.5A	1.1b	1.5 <sup>a</sup>	0.09	<0.001	0.002	0.90
Acid detergent fiber	0.4B	0.7A	0.4b	0.7 <sup>a</sup>	0.05	<0.001	<0.001	0.92
Cellulose	0.3B	0.6A	0.4b	0.5 <sup>a</sup>	0.04	<0.001	<0.001	0.85

<sup>A,a</sup> Means followed by uppercase letters in each line and lowercase letters in each column differ according to Tukey's multiple-mean test ( $P \leq 0.05$ ); <sup>1</sup> Standard error; \*Consumption in g/metabolic body weight g/day ( $\text{kg}^{0.75}$ )

## Results

Ewe lambs fed diets formulated for early maturity consumed more EE, non-fibrous carbohydrates, and TDN (Table 1). In contrast, ewe lambs fed late-maturity diets (Table 2) consumed more CP and fibrous fractions. Restricting CP and TDN resulted in reduced non-fiber and greater fibrous component consumption.

Nutritional levels geared to early-carcass finishing resulted in feeds with 7.11% greater DMD compared to those geared to late maturity (Table 3). This influenced digestibility coefficients of EE, cellulose and non-fibrous carbohydrates in early-maturity diets. Restricting CP and TDN by 15% relative to that recommended by NRC resulted in decreased DM and EE but increased NDF.

Ewe lambs fed late-maturity diets in general ingested more N (IN), excreted more N (EN) and retained more N (RN) as well as had greater RN/IN and EN/IN ratios (Table 4). The FN/IN and EN/IN ratios were greater for ewe lambs on early-maturity diets. Restricting CP and TDN by 15% resulted in IN, FN, RN and RN/IN ratio decreases and increases in EN and UN/IN and EN/IN ratios.

Rumin liquid pH was greater in ewe lambs fed early-maturity diets without restrictions (Table 5). Lower N-NH<sub>3</sub> was apparent in ewe lambs consuming early-maturity diets without CP and TDN restriction. For those fed late-maturity diets, when nutrients were restricted, values were inferior to those fed early-maturity diets.

There were no differences in blood TN and albumin concentrations between ewe lambs fed early- and late-maturity diets. Except for urea which was greater in which case the order was reversed, all other blood metabolite levels were

**Table 3** Coefficients of digestibility (%) of nutrients consumed by Santa Inês ewe lambs fed diets formulated according to NRC (2007) for early- and late-maturity with 0 or 15% crude protein and total digestible nutrient restrictions

	Maturity (M)		Restriction (R)		SE <sup>1</sup>	P-value		
	Early	Late	0%	15%		M	R	M*R
Dry matter	67.4A	62.6B	67.2a	62.7b	1.0	0.004	0.007	0.05
Organic matter	83.4	81.4	83.4	80.9	0.8	0.250	0.11	0.16
Crude protein	54.0B	67.4A	61.5	59.9	2.0	<0.001	0.50	0.26
Ether extract	76.7A	49.7B	71.3a	55.1b	3.6	<0.001	<0.001	0.37
Neutral detergent fiber	44.2B	49.9A	45.3b	48.8a	1.8	<0.001	0.004	0.13
Cellulose	70.2A	61.8B	65.6	66.7	1.0	<0.001	0.63	0.83
Non-fibrous carbohydrates	88.9A	84.6B	87.5	87.5	1.1	0.044	0.47	0.05

<sup>A,a</sup> Means followed by uppercase letters in each line and lowercase letters in each column differ according to Tukey's multiple-mean test ( $P \leq 0.05$ ); <sup>1</sup> Standard error

greater in ewe lambs fed early-maturity diets compared to late-maturity diets (Table 6). Blood urea was also greater when there was no CP and TDN restriction according to NRC (2007).

Diets formulated for late maturity resulted in greater ruminant microbial N and protein synthesis, as well as ruminant degradable and non-degradable N and metabolizable protein compared to those formulated for early maturity (Table 7). Restricting CP and TDN 15% reduced rumen-bypass protein and metabolizable protein intake compared to unrestricted diets.

## Discussion

Reducing CP and TDN 15% compared to that recommended by NRC (2007) for ewe lambs resulted in less soluble nutrient

consumption as a result of greater fiber ingestion (Table 2). Dry matter intake, however, was not limited as NRC (2007) recommends a minimum 74 g/metabolic body size (MBW) for growing ewe lambs. Reducing CP and TDN 15% relative to what NRC (2007) recommends, therefore, could be viable for Santa Inês ewe lambs. Growing animals have elevated protein requirements as a result of greater protein turnover observed during early growth, a reflection of greater muscle deposition (Owens et al., 1993; CSIRO, 2007). That possibly contributed to increased DMI, especially for ewe lambs consuming diets formulated for late maturity, with greater CP content (Table 2).

Restricting CP by 15% in diets fed to early-maturity ewe lambs did not allow ewe lambs to consume the minimum 10.3 g/MBW recommended by NRC (2007). As such, restricting CP and TDN in diets fed to early-maturity ewe lambs could negatively affect their long-term growth. Such was the case for Costa et al. (2020) who, when evaluating the growth

**Table 4** Nitrogenous compound balance in Santa Inês ewe lambs fed diets formulated according to NRC (2007) for late or early maturity with 0 or 15% crude protein and total digestible nutrient restriction

	Maturity (M)		Restriction (R)		SE <sup>1</sup>	P-value		
	Early	Late	0%	15%		M	R	M*R
g/day of N								
Ingested (IN)	13.8B	23.2A	20.7a	16.3b	1.3	<0.001	0.005	0.01
Fecal (FN)	6.1	7.1	7.2a	6.1b	0.3	0.11	0.05	0.21
Urinary (UN)	1.2B	2.8A	1.6b	2.4a	0.2	<0.001	<0.001	1.00
Excreted (EN)	7.4B	9.9A	8.8	8.5	0.4	<0.001	0.61	0.18
Retained (RN)	6.2B	13.7A	11.9a	8.0b	1.2	<0.001	<0.001	0.06
Ratios								
RN/IN	0.5B	0.6A	0.5a	0.5b	0.0	0.009	0.002	0.40
FN/II	42.9A	29.3B	36.4	3.8	1.9	<0.001	0.778	0.58
UN/IN	9.1B	13.0A	7.0	14.6a	1.0	0.006	<0.001	0.27
EN/IN	53.9A	43.2B	43.9b	53.2a	2.0	0.002	0.005	0.68

<sup>A,a</sup> Means followed by uppercase letters in each line and lowercase letters in each column differ according to Tukey's multiple-mean test ( $P \leq 0.05$ ); <sup>1</sup> Standard error

**Table 5** Rumin liquid pH and ammonium N of Santa Inês ewe lambs fed diets formulated according to NRC (2007) for late or early maturity with 0 or 15% crude protein and total digestible nutrient restriction

Restriction (R)	Maturity (M)		Mean	SE <sup>1</sup>	P-value		
	Early	Late			M	R	M*R
pH							
0%	6.7	6.8	6.8b	0.05	<0.0001	<0.0001	0.22
15%	6.8	7.2	7.0a				
Mean	6.7B	7.0A					
N-NH <sub>3</sub>							
0%	16.6Bb	26.5Aa	21.6	0.93	0.062	0.96	0.001
15%	23.2Aa	19.8Ab	21.5				
Mean	19.9	23.2					

<sup>A,a</sup> Means followed by uppercase letters in each line and lowercase letters in each column differ according to Tukey's multiple-mean test ( $P \leq 0.05$ ); <sup>1</sup> Standard error

curves of the same animals studied in our trial, reported lower growth rates when Santa Inês lambs were fed early-maturity diets with 15% CP and TDN. Oliveira et al. (2020), working with growing (4 to 8 months) Morada Nova lambs observed no CP intake reduction when they restricted CP and TDN by 15% compared to that recommended by NRC (2007).

Greater non-fibrous carbohydrate and EE intakes (Table 2) reflected greater TDN intake in ewe lambs fed early-maturity diets with no nutrient restrictions. For late-maturing ewe lambs, NRC (2007) recommends 58.2 g/MBW and 69.8 g/MBW for early maturity. It is possible that, to achieve maturity more rapidly compared to other breeds, Santa Inês ewe lambs may have been more efficient at utilizing dietary energy. Greater EE and non-fibrous carbohydrate digestibilities were observed in diets fed to early-maturity ewe lambs, thereby contributing to greater DMD (Table 3). If the intention is to produce replacement ewes, IE feeding them beyond 8 months of age, Costa et al. (2020) suggested using early-maturity diets without nutrient restrictions which did not negatively affect ADG and DMI.

Feeding high-concentrate diets, such as early-maturity diets without nutrient restrictions (Table 2), can cause metabolic distress such as ruminal lactic acidosis, with resulting decreases in DMI and ADG (Rogério et al., 2018). The NDF intakes in our trial remained within those recommended by Mertens (1994), 0.8 to 1.2% of lamb BW, indicating low risk for feeding disorders. Restricting diet CP and TDN 15% reduced DMD 6.66%, explained by the increase in bulk/concentrate ratios and plant-wall components such as lignin (Tables 1 and 3). However, reducing CP and TDN by 15% did not reduce OM, CP, or non-fibrous carbohydrate digestibilities compared to unrestricted diets. This corroborates what Costa et al. (2020) reported for 15% CP and TDN reduction that was not detrimental to ewe lamb feed efficiency vis-à-vis unrestricted diets up to 8 months of age.

The late-maturity diet resulted in greater EN as a result of greater CP intake that increased rumen ammonium N and blood urea (Tables 4, 5, and 6). The plasma urea levels we observed fall within the optimum 20 to 60 mg/dL recommended by González and Silva (2006). Creatine values also fell

**Table 6** Blood metabolites of Santa Inês ewe lambs fed diets formulated according to NRC (2007) for late or early maturity with 0 or 15% crude protein and total digestible nutrient restriction

	Maturity (M)		Restriction (R)		SE <sup>1</sup>	P-value		
	Early	Late	0%	15%		M	R	PN*R
g/dL								
Total protein	6.4	6.5	6.4	6.4	0.1	0.6	0.88	0.92
Albumin	2.7	2.8	2.9	2.6	0.1	0.76	0.3	0.68
Mg/dL								
Urea	23.5B	63.1A	47.5a	39.1b	5.0	<0.001	0.01	0.26
Creatine	1.0A	0.8B	0.9	0.9	0.0	0.05	0.40	0.51
Glucose	70.6A	62.2B	69.4	63.4	2.3	0.04	0.15	0.27
Triglycerides	27.2A	20.2B	23.7	23.0	1.4	0.01	0.93	0.10
Cholesterol	60.7A	42.2B	51.8	51.1	3.9	0.01	0.98	0.26

<sup>A,a</sup> Means followed by uppercase letters in each line and lowercase letters in each column differ according to Tukey's multiple-mean test ( $P \leq 0.05$ ); <sup>1</sup> Standard error

**Table 7** Ruminal nitrogenous metabolites of Santa Inês ewe lambs fed diets formulated according to NRC (2007) for late or early maturity with 0 or 15% crude protein and total digestible nutrient restriction

	Maturity (M)		Restriction (R)		SE <sup>1</sup>	P-value			
	Early	Late	0%	15%		M	R	M*R	
g/day synthesized									
Microbial nitrogen	3.6B	6.5A	5.2	4.9	0.5	0.02	0.37	0.18	
Microbial protein	22.7B	40.9A	32.7	30.8	3.1	0.02	0.37	0.18	
g/day of Total digestible nutrients synthesized									
Microbial nitrogen	7.2B	13.7A	9.8	11.1	1.5	0.03	0.06	0.82	
Microbial protein	44.9B	85.9A	61.2	69.6	9.7	0.03	0.06	0.82	
g/day of dry matter									
Rumin-degraded protein	25.3B	43.4A	36.1	32.5	3.4	0.02	0.42	0.07	
Rumin non-degraded protein	59.1B	111.7A	100.1a	70.7b	11.5	<0.001	0.002	0.07	
Metabolizable protein	64.8B	109.5A	98.2a	76.2b	8.1	<0.001	<0.001	0.07	

<sup>A,a</sup> Means followed by uppercase letters in each line and lowercase letters in each column differ according to Tukey's multiple-mean test ( $P \leq 0.05$ ); <sup>1</sup> Standard error

within the 0.4 to 1.8 mg/dL recommended by Varanis (2018). According to Van Soest (1994), however, urea values over 30 mg/dL, such as those measured in ewe lambs fed late-maturity diets, can cause greater EN and renal dysfunction. In addition, greater N losses can result in inefficient energy consumption with negative environmental effects (Spek et al., 2013).

The absorption of protein metabolites that we measured in ewe lambs supports the use of early- and late-maturity diets, with or without nutrient restrictions. Albumin values fell within the 6.0 to 7.9 g/dL total proteins normal for sheep (Kaneco et al., 2008). The lowest pH value we measured was 6.7, indicating adequate conditions for ruminal fermentation process in all experimental diets (Sung et al., 2007). The lowest ruminal fluid N-NH<sub>3</sub> that we measured, 16.6 mg/dL, occurred in ewe lambs fed late-maturity diets, greater than the minimum reported by Detmann et al. (2014) required for microbial protein synthesis, possibly as a result of greater CP intake (Table 2). Microbial proteins represent 60 to 85% of the protein required for ruminant maintenance (Timmermans Jr. et al., 2000). Wassie et al. (2019) suggested different nutrient requirements for cattle (bovines) compared to other ruminants and pointed out that, as CP intake increases, so does ruminal microbial protein synthesis. In our trial, the greater quantity of rumin non-degradable protein consumed by lambs implied greater metabolizable protein in lambs fed late-maturity diets (Table 7).

Reducing dietary CP and TDN 15% should be undertaken cautiously when feeding ewe lambs. According to NRC (2007), the metabolizable protein levels we measured were below those recommended for both early- and late-maturity diets. Costa et al. (2020), however, did not measure any reduction in ewe lamb animal performance when fed either diet up to 8 months of age but warned that ADG may be compromised beyond 8 months of age.

## Conclusions

Depending on production system goals, two different diets are indicated. When the objective is to finish carcasses quickly, an early-maturity diet without CP or TDN restriction works best. When the objective is to eventually produce replacement ewes, the late-maturity diet with CP and TDN, restricted by 15% compared to NRC (2007) tables, is recommended.

**Author contribution** Conceptualization: CSC. Data curation: CSC and FGSA. Formal analysis: CSC. Funding acquisition: MCPR, RCFFP, JNMN, ALF. Investigation: CSC and FGSA. Methodology: MCPR, ALF, FSM, PGP, JPAR, CSC, and FGSA. Project administration: MCPR. MCPR, ALF, FSM, and JNMN. Writing-original draft: CSC. Writing-review and editing: MCPR and JPM.

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## Declaration

**Ethics approval** All procedures were approved by the Committee on Ethics in the Use of Animals of Embrapa Caprinos e Ovinos (CEUA), protocol n° 001/2017.

**Conflict of interest** The authors declare no competing interests.

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