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Effect of dietary reduction and sex class on nutrient digestibility, nitrogen balance, excreted purine derivatives and infrared thermography of hair lambs

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Abstract

An experiment was conducted to evaluate whether dietary reduction and sex class affect nutrient intake, digestibility, purine derivative (PD) excretion and heat tolerance coefficient in lambs. Thirty-five hair lambs (14.5 ± 0.89 kg initial body weight (BW), 2 months old) were used in a completely randomized study with a 3 × 3 factorial arrangement, three sex classes (11 intact males, 12 castrated males and 12 females) and three levels of feeding (*ad libitum*, 300 and 600 g/kg/dry matter (DM) feed restriction) for 120 days. Intact and castrated males showed higher intakes of DM and neutral detergent fibre corrected for ash and protein (NDFap) than females. At 300 g/kg/DM feed restriction, NDFap digestibility was lower in intact males than in other classes; however, no differences were found between classes when subjected to *ad libitum* feeding or 600 g/kg/DM. The basal endogenous nitrogen and endogenous urinary losses were highest in intact males. Allantoin, uric acid and PD excretion, as well as PD absorption and microbial protein production were lowest in the animals subjected to 600 g/kg/DM feed restriction. Microbial protein synthesis (MPS) was highest in animals subjected to 600 g/kg/DM feed restriction. The lowest temperatures were observed in animals subjected to 600 g/kg/DM feed restriction. The heat tolerance coefficient was highest in animals subjected to 600 g/kg/DM feed restriction. In conclusion, feed restriction reduced the time spent on feeding and rumination but increased the digestibility of DM. The restriction level of 600 g/kg/DM maximized MPS and infrared thermography indicated an elevated heat tolerance coefficient.

Introduction

Hair sheep show traits that are desirable for lamb production, especially in extensive production systems (Costa *et al*., 2013) and represent an important genetic resource for producing lambs (McManus *et al*., 2015). These animals are usually bred in the Caatinga biome (Maciel *et al*., 2015), which exhibits seasonal fluctuations in feed availability during the year. Farmers' dependence on the availability of feed from nature has been the primary problem facing hair sheep production in semi-arid regions.

The ability of ruminants to undergo metabolic and endocrine adaptation to feeding restrictions depends on species and physiological characteristics (Rodrigues *et al*., 2016), as well as age, sex and breed (de Araújo *et al*., 2017). In addition to nutritional variables, which are important for adjustments in animal management practices, it is necessary to be concerned with the physiological aspects of animals bred in these regions. Thermal measurements taken in production systems allow verification of the animals' welfare and the impacts of specific alimentary plans on thermoregulatory processes (Knizkova *et al*., 2007; McManus *et al*., 2015).

Given the many functions that nutrients fulfil in animals' bodies, adequate protein and energy supplies during growth are of paramount importance (Rodrigues *et al*., 2016; Pereira *et al*., 2017). Knowledge of the effect of feed restriction on growing hair sheep (Pereira *et al*., 2014) is of global importance because it would allow the development of better food strategies and would reduce costs in the formulation of diets (Costa *et al*., 2013) and environmental perspectives. Therefore, the hypothesis investigated in the current paper was that feed restriction could affect intake, digestibility, microbial efficiency synthesis and heat tolerance. In this context, the current study was conducted to evaluate the impact of dietary reduction on nutrient intake and digestibility, feeding behaviour, urinary excretion of purine derivatives

(PD), nitrogen balance and rectal and superficial temperatures in growing Morada Nova lambs.

Materials and methods

Animals, treatments and general procedures

Thirty-five hair lambs (14.5 ± 0.89 kg initial BW, 2 months old) were used in a completely randomized study with a 3 × 3 factorial arrangement with three sex classes (11 intact males, 12 castrated males and 12 females) and three levels of feeding (*ad libitum*, 300 and 600 g/kg/dry matter (DM) feed restriction for 120 days). The lambs were placed in individual pens equipped with feeders and water troughs.

All lambs were fed a similar diet throughout the experimental trial. A total mixed ration (TMR) (Table 1) was formulated to meet the nutritional requirements of late-maturity lambs as defined by the NRC (2007) with a gain of 150 g/day. In the formulation of the TMR, Tifton 85 hay (*Cynodon* sp.) was used as roughage (600 g/kg/DM). The lambs were fed twice daily, at 07.30 and 16.00 h, allowing for up to 100 g/kg/DM refusals only for animals fed *ad libitum*, and their intake was recorded on the basis of the daily feed supply. Before each morning feeding, the refusals from the previous day's feedings for each animal fed *ad libitum* were removed and weighed to calculate the intake and feeding levels of the lambs of each sex class submitted to 300 and 600 g/kg/DM feed restriction. Every 15 days, the lambs were weighed in the morning (at 07.00 h, before feeding) to obtain their body weight (BW).

Digestibility trial, collection of urine and determination of microbial protein synthesis

The digestibility trial was carried out indirectly using indigestible neutral detergent fibre (iNDF) as to estimate faecal DM excretion. Every 15 days, at specific times (08.00 h on the first day, 12.00 h on the second day and 04.00 h on the third day), faeces were collected from the animals' rectal ampulla. Faecal samples, refusals, concentrate and Tifton 85 hay were incubated *in situ* over a period of 240 h in the rumen of a cow receiving an experimental feed. After, the bags were washed in water until they became clear (Van Soest *et al.*, 1991), and the residue was weighed and considered to be the iNDF. An estimate of the total digestible nutrients (TDN) was calculated according to Weiss (1993) as follows:

$$TDN = CP_d + NFC_d + NDFap_d + EE_d \times 2{\cdot}25$$

where CP is the crude protein, NFC is the non-fibre carbohydrates, NDFap is the neutral detergent fibre corrected for ash and protein and EE is the ether extract. Subscript d means digestible.

Spot urine samples were collected every 20 days, approximately 4 h after the morning feeding, during spontaneous urination, used for analysis of PD to estimate microbial protein synthesis (MPS). For collection of urine from males, plastic collectors adapted to the animal's body were used. Disposable urethral catheters were used to collect urine from females. Samples of urine (5 ml) were collected, diluted with 45 ml of a solution of 0.018 mmol sulphuric acid (H_2SO_4), and stored at −20 °C. The concentration of creatinine in the urine was determined using a commercial kit (Labtest, Lagoa Santa, MG, Brazil) to estimate the urinary volume.

The analysis of allantoin in the urine was performed using a colorimetric method (Fujihara *et al.*, 1987). The concentration of uric acid was evaluated using an enzymatic colorimetric test with clearing factor lipase (Barham and Trinder, 1972; Fossati *et al.*, 1980), and the creatinine concentration was analysed using the alkaline picrate method (Henry *et al.*, 1974). To estimate microbial purine derivative absorption (AbsPD), the following equations were used, proposed by Chen and Gomes (1992):

$$Y = 0{\cdot}84X + [(0{\cdot}150\ BW)^{0{\cdot}75} e^{(-0{\cdot}25X)}] \quad (1)$$

where Y is expressed in mmol/day, X is the AbsPD (mmol/day), 0.84 represents the recovery of absorbed purines as PD in urine, and the component within brackets represents the endogenous contribution, which decreases as exogenous purines become available for utilization by the lambs.

$$MN(g/day) = 70X\ (mmol/d)/(0.116'0.83'1000) = 0.727X \quad (2)$$

where MN is microbial nitrogen (g N/day), assuming that the N concentration of purine is 70 mg N/mmol, the ratio of purine N to total N in mixed ruminal microbes is 11.6:100, and the digestibility of microbial purines is 0.83. The MN values were multiplied by a factor of 6.25 to obtain microbial crude protein (CPmic). Subsequently, the MPS was calculated using the following equation:

$$MPS = CPmic/intake\ of\ TDN$$

The amounts of N intake (g/day) and N excreted in faeces and urine were considered for calculation of the N balance (NB). Nitrogen retention was calculated as the difference between NB and basal endogenous nitrogen loss (BEN), assuming the endogenous tissue and dermal N losses to be 0.35 and 0.018 metabolic weight, respectively. The equation to calculate the BEN was:

$$BEN\ (g/day) = (0{\cdot}018 + 0{\cdot}35) \times BW^{0{\cdot}75}$$

The value of nitrogen retained (NR) was expressed as NR = NB −BEN. Endogenous urinary losses (EUL) were calculated from the equation adapted for sheep by ARC (1980):

$$EUL\ (g/day) = 0{\cdot}147 \times BW + 3{\cdot}375$$

Ingestive behaviour and infrared thermography

Ingestive behaviour of lambs was characterized by measuring feeding time (TAL, h/day) and rumination time (TRU, h/day) at 10 min intervals for 24 h, every 20 days, according to the methodology proposed by Johnson and Combs (1991). To evaluate thermal stress on the animals, rectal temperature (RT) was measured at 09.00 and 14.00 h with a digital thermometer inserted near the rectal ampulla of the animal, at a depth of approximately 3.5 cm. The Iberia heat tolerance test was used to determine the heat tolerance coefficient, a measure of the adaptability of the animals, according to the following equation:

$$HTC = 100 - [18\,(RT - 39{\cdot}1)]$$

where HTC = heat tolerance coefficient; 100 = maximum efficiency to maintain body temperature at 39.1 °C; 18 = constant; RT =

Table 1. Ingredient and chemical composition of experimental ration

Ingredient	g/kg dry matter (DM)			
Tifton 85 grass hay	600.0			
Ground maize	327.2			
Soybean meal	63.0			
Dicalcium phosphate	0.6			
Mineral premix[a]	9.2			
Chemical composition (g/kg DM)	**Total ration**	**Tifton 85 grass hay**	**Ground maize**	**Soybean meal**
Dry matter	908.0	913.0	892.0	910.0
Crude protein	169.0	172.0	103.0	509.0
Ether extract	30.8	25.6	43.2	19.3
Ash	61.9	73.4	13.3	65.9
Neutral detergent fibre	439.0	668.0	113.0	135.0
NDFap	418.0	645.0	97.9	110.0
Acid detergent fibre	202.0	318.0	26.3	102.0
Total carbohydrates	738.0	752.0	842.0	408.0
Non-fibre carbohydrates	320.0	58.3	728.0	273.0
Total digestible nutrients	674.0	–	–	–

NDFap, neutral detergent fibre corrected for ash and protein.
[a]Composition, 1 kg of premix: calcium 225–215 g; phosphor 40 g; sulphur 15 g; sodium 50 g; magnesium 10 g; cobalt 11 mg; iodine 34 mg; manganese 1800 mg; selenium 10 mg; zinc 2000 mg; iron 1250 mg; copper 120 mg; fluor 400 mg; vitamin A 37.5 mg; vitamin D3 0.5 mg and vitamin E 800 mg.

average RT (°C) based on readings at 09.00 and 14.00 h; and 39.1 °C = average RT considered normal for sheep (Reece *et al.*, 2015).

Thermal scanning of lambs was performed using an infrared camera (FLIR, 620 series; FLIR Systems Co. Ltd., St Leonards, New South Wales, Australia). Temperatures were measured in the regions of the rib and the flank from the right and left sides and in the pectoral region of the animal (Fig. 1). The measurements were taken every 20 days for three consecutive days, totalling 3780 images at the end of the experimental period, which were analysed using Quick Report® software. For the analysis of temperatures, rectangles were drawn using the 'Area' tool, which defines the mean temperature of a delimited area.

The air temperature (TA) and relative humidity (RH) were measured every 15 min by means of two data loggers placed on the premises at the collection point for the physiological parameters. The temperature–humidity index (THI) was obtained by the formula described by Buffington *et al.* (1983):

$$\text{THI} = 0.8\ \text{Tdb} + \text{RH}\ (\text{Tdb} - 14.3)/100 + 46.3$$

where Tdb is the dry bulb temperature. The mean temperature during the experimental trial was 28.8 °C (morning: 27.6 °C; afternoon: 30.0 °C), and the RH was 77.3% (morning: 78.1%; afternoon: 64.9%).

Analysis and calculations

Samples of the ingredients, orts and faeces were dried in a forced air oven at 55 °C for 72 h, ground to pass through a 1 mm screen (Wiley Mill, Arthur H. Thomas, Philadelphia, PA, USA) and subsequently analysed for DM (AOAC, 1990; method number 967.03), ash (AOAC, 1990; method number 942.05), CP (AOAC, 1990; method number 981.10), EE (AOAC 1990; method number 920.29), acid detergent fibre (AOAC 1990; method number 913.18), neutral detergent fibre (NDF, Van Soest *et al.*, 1991) and fibrous carbohydrates (Sniffen *et al.*, 1992). The NDF analysis was performed using thermostable α-amylase without sodium sulphite and was corrected for residual ash (Mertens, 2002) and residual nitrogenous compounds (Licitra *et al.*, 1996). The quantity of NFC was calculated using an equation described by Hall (2000):

$$\text{NFC} = 1000 - (\text{NDFap} + \text{CP} + \text{EE} + \text{ash})$$

Total carbohydrate content (TC) was calculated according to Sniffen *et al.* (1992):

$$\text{TC} = 1000 - (\text{CP} + \text{EE} + \text{ash})$$

Statistical analysis

The data were subjected to analysis of variance using the GLM procedure in SAS version 9.0 (SAS® Inst. Inc., Cary, NC, USA) with the following mathematical model:

$$Y_{ijk} = \mu + S_i + R_j + S_i \times R_j + \varepsilon_{ijk}$$

where Y_{ijk} is the dependent or response variable measured in animal or experimental unit 'k' of sex class 'i' at feed restriction 'j'; μ is the population mean or global constant; S_i is the effect of sex class 'i'; R_j is the effect of feed restriction 'j'; $S_i \times R_j$ is the interaction between effects of sex class 'i' and feed restriction 'j'; and ε_{ijk} is the unobserved random error. Tukey–Kramer's test was used to compare the means at $P < 0.05$ and the same criterion was adopted for interactions between the effects of sex and feed restriction.

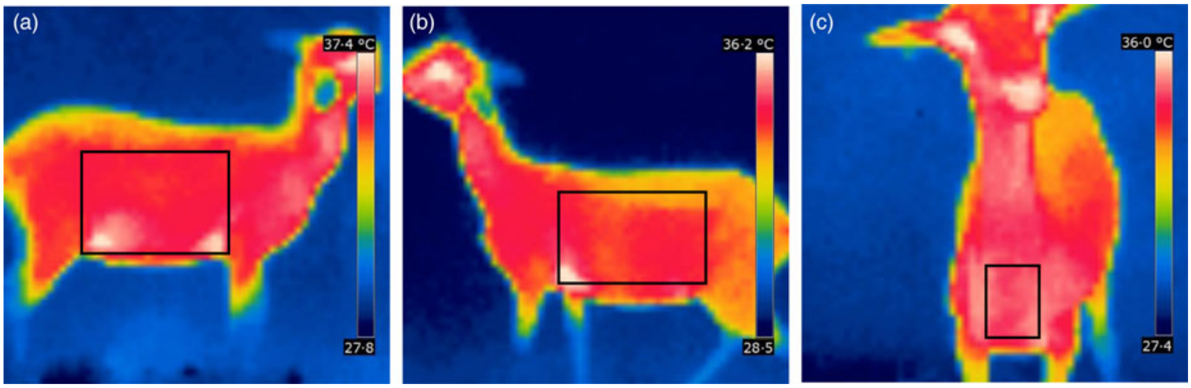

Fig. 1. Thermographic images and temperature collection areas of Morada Nova lambs. Regions of the right side (A), left side (B) and pectoral region (anterior aspect) (C).

Thermographic results were evaluated according to a mathematical model:

$$Y_{ijkl} = \mu + S_i + R_j + T_k + S_i \times R_j + S_i \times T_k + R_j \times T_k + S_i \times R_j \times T_k + \varepsilon_{ijkl}$$

where Y_{ijkl} is the dependent or response variable measured in the animal or experimental unit '*l*' of sex class '*i*' at feed restriction '*j*' and period '*T*'; μ is the population mean or global constant; S_i is the fixed effect of sex class '*i*'; R_j is the fixed effect of feed restriction '*j*'; T_k is the fixed effect of period '*k*'; $S_i \times R_j$ is the interaction between effects of sex class '*i*' and feed restriction '*j*'; $S_i \times T_k$ is the interaction between sex class '*i*' and period '*k*'; $R_j \times T_k$ is the interaction between feed restriction '*j*' and period '*k*'; $S_i \times R_j \times T_k$ is the interaction of sex class '*i*', feed restriction '*j*' and period '*k*'; and ε_{ijkl} is the unobserved random error. The Tukey–Kramer test was used to compare the means at $P < 0.05$ and the same criterion was adopted for interactions among the effects of sex, feed restriction and period.

Results

There were effects of sex and feed restriction ($P < 0.05$) on nutrient intake (Table 2). Intact and castrated males consumed more DM and NDFap (%BW and $BW^{0.75}$) than females ($P < 0.05$). Neither eating time nor ruminating time was affected by sex. Feed restriction influenced nutrient intake significantly ($P < 0.05$). Dry matter intake, NDFap intake (%BW and $BW^{0.75}$) and eating time were highest in lambs with *ad libitum* intake ($P < 0.05$), followed by lambs subjected to 300 g/kg/DM feed restriction. Time spent ruminating was similar for animals subjected to *ad libitum* feeding and 300 g/kg/DM feed restriction.

There was no influence of sex class on the CP, EE or NFC digestibility coefficient (Table 3). Sex class influenced DM digestibility ($P < 0.05$), which was highest in castrated males. The digestibility of DM and CP was lower ($P < 0.05$) in animals fed *ad libitum* than in animals subjected to 300 or 600 g/kg/DM feed restriction ($P < 0.05$). Ether extract digestibility ($P < 0.05$) was highest in animals subjected to 300 g/kg/DM feed restriction.

There were interaction effects ($P < 0.05$) of sex and feed restriction on OM, NDFap and TC digestibility (Table 4). At 300 g/kg/DM feed restriction, the digestibility of OM and NDFap was lowest in intact males; however, intact males, castrated males and females did not differ when subjected to *ad libitum* feeding or 600 g/kg/DM feed restriction. Total carbohydrate digestibility was highest at 600 g/kg/DM ($P < 0.05$) in all sex class.

Nitrogen retention, purine derivatives and microbial efficiency

Nitrogen intake, faecal nitrogen, BEN and EUL were higher in intact males ($P < 0.05$) (Table 5). Feed restriction influenced the overall balance of *N* compounds ($P < 0.05$) and lambs subjected to 600 g/kg/DM feed restriction excreted those compounds at lower levels than lambs under the other feeding conditions.

Urine output and creatinine excretion were not influenced by sex class or feed restriction (Table 5). Sex class did not affect allantoin, xanthine and hypoxanthine, AbsPD, total PD, CPmic or MPS, but did affect uric acid excretion ($P < 0.05$); the values of the latter were higher in males than in females. Allantoin, uric acid, AbsPD, total PD and CPmic production were lowest in the animals subjected to 600 g/kg/DM feed restriction ($P < 0.05$). The efficiency of MPS was highest in the animals subjected to 600 g/kg/DM feed restriction ($P < 0.05$).

Thermographic temperatures

Surface temperatures on the right and left sides (Fig. 1) and the front pectoral region, as well as RT, were influenced by feed restriction and period ($P < 0.05$, Table 6). The lowest temperatures ($P < 0.05$) were observed in animals subjected to 600 g/kg/DM feed restriction. Decreased temperatures were also recorded in the morning period ($P < 0.05$). HTC was highest in animals subjected to 600 g/kg/DM feed restriction ($P < 0.05$).

There was an interaction effect ($P < 0.05$) of sex class and feed restriction on surface temperature on the right side of the sheep (Table 7), whose lowest morning values were in females subjected to 600 g/kg/DM feed restriction and whose lowest afternoon values were in females and castrated males subjected to 600 g/kg/DM feed restriction.

Discussion

Digestibility in the rumen results from the competition between digestion and passage rates (Dhanoa *et al.*, 1995), and passage rate is positively correlated with intake of DM (Van Soest, 1994). The greatest protein digestibility coefficients for animal

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Table 2. Effects of sex class and feed restriction on nutritional variables in hair lambs (means and ± s.d.)

	Sex class (*S*)[a]			Feed restriction (FR)[b]				*P*-value		
Item	IM	CM	*F*	AL	300 g/kg/DM	600 g/kg/DM	s.e.m	*S*	FR	*S* × FR
Intake (g/day)										
Dry matter	666 ± 218.1	594 ± 201.2	517 ± 171.2	804 ± 121.1	619 ± 65.2	355 ± 37.4	33.6	<0.001	<0.001	0.371
Organic matter	625 ± 206.2	557 ± 189.1	485 ± 161.4	755 ± 114.3	580 ± 61.1	333 ± 35.1	31.6	<0.001	<0.001	0.352
Crude protein	113 ± 37.9	101 ± 34.9	88 ± 29.3	137 ± 21.4	104 ± 10.0	60 ± 6.3	5.8	<0.001	<0.001	0.341
Ether extract	21 ± 6.9	18 ± 6.3	16 ± 5.4	25 ± 3.9	19 ± 2.0	11 ± 1.2	1.1	<0.001	<0.001	0.372
NDFap	275 ± 82.4	246 ± 75.6	214 ± 64.2	317 ± 48.2	266 ± 28.0	153 ± 16.1	12.7	<0.001	<0.001	0.455
Total carbohydrates	491 ± 161.2	438 ± 148.2	381 ± 126.1	592 ± 88.5	456 ± 48.1	262 ± 27.6	24.7	<0.001	<0.001	0.364
NFC	216 ± 74.6	193 ± 68.5	168 ± 59.5	276 ± 37.5	191 ± 18.6	110 ± 10.7	12.1	<0.001	<0.001	0.253
TDN	425 ± 113.2	402 ± 126.2	348 ± 105.2	505 ± 71.9	418 ± 36.4	251 ± 30.0	19.5	0.002	<0.001	0.885
Performance (g/day)										
Average daily gain	106 ± 50.6	72 ± 42.1	43 ± 30.1	116 ± 42.2	81 ± 25.9	23 ± 19.0	2.3	<0.001	<0.001	0.030
Intake (g/kg BW)										
Dry matter	34 ± 0.65	33 ± 0.96	31 ± 0.65	39 ± 0.25	34 ± 0.28	25 ± 0.17	0.11	<0.001	<0.001	0.261
NDFap	14 ± 0.26	14 ± 0.25	13 ± 0.24	16 ± 0.11	15 ± 0.13	11 ± 0.08	0.04	<0.001	<0.001	0.273
Intake (g/kg $BW^{0.75}$)										
Dry matter	710 ± 16.0	680 ± 16.3	620 ± 14.9	840 ± 6.6	700 ± 5.7	480 ± 3.5	2.6	<0.001	<0.001	0.402
NDFap	300 ± 6.3	280 ± 6.2	260 ± 5.7	330 ± 2.8	330 ± 2.6	210 ± 1.6	0.97	<0.001	<0.001	0.402
Ingestive behaviour (h/day)										
Time spent eating	3.0 ± 1.3	3.0 ± 1.5	3.0 ± 1.5	4.4 ± 0.48	2.4 ± 0.49	1.3 ± 0.14	0.23	0.523	<0.001	0.761
Time spent ruminating	7.0 ± 1.6	7.0 ± 1.5	7.0 ± 1.7	8.2 ± 0.60	7.0 ± 1.1	5.1 ± 0.66	0.26	0.111	<0.001	0.532

s.d., standard deviation; s.e.m, standard error of the mean; DM, dry matter; NDFap, neutral detergent fibre corrected for ash and protein; NFC, non-fibre carbohydrates; TDN, total digestible nutrients; BW, body weight.
[a]Sex class: IM, intact males; CM, castrated males; F, females.
[b]Feed restriction: *ad libitum* (AL) or 300 or 600 g/kg/DM reduction of the AL intake.

Table 3. Effect of sex class and feed restriction on digestibility coefficients in hair lambs (means and ± s.d.)

	Sex class (*S*)[a]			Feed restriction (FR)[b]				*P*-value		
	IM	CM	*F*	AL	300 g/kg/DM	600 g/kg/DM	s.e.m	*S*	FR	*S* × FR
Dry matter (g/kg)	66 ± 5.9	69 ± 4.2	69 ± 3.7	63 ± 4.1	68 ± 4.1	71 ± 1.9	0.79	0.041	<0.001	0.062
Crude protein (g/kg DM)	70 ± 5.0	73 ± 4.2	73 ± 3.4	68 ± 4.3	73 ± 4.0	75 ± 1.9	0.73	0.050	<0.001	0.173
Ether extract (g/kg DM)	59 ± 4.7	61 ± 4.5	62 ± 2.3	58 ± 4.2	63 ± 4.5	61 ± 3.2	0.67	0.163	0.009	0.092
Non-fibrous carbohydrates (g/kg DM)	84 ± 3.6	86 ± 2.7	85 ± 2.4	84 ± 3.4	85 ± 3.0	86 ± 1.8	0.49	0.572	0.175	0.865

s.d., standard deviation; s.e.m, standard error of the mean.
[a]Sex class: IM, intact males; CM, castrated males; F, females.
[b]Feed restriction: *ad libitum* (AL) or 300 or 600 g/kg/DM reduction of the AL intake.

Table 4. Interaction effects of sex class and feed restriction on nutrient digestibility coefficients in hair lambs (means and ± s.d.)

		Feed restriction (FR)[b]				*P*-value		
Item (g/kg)	Sex class (*S*)[a]	AL	300 g/kg/DM	600 g/kg/DM	s.e.m	*S*	FR	*S* × FR
OM	IM	62 ± 2.6	66 ± 3.1	74 ± 1.9		0.032	<0.001	0.050
	CM	66 ± 4.6	72 ± 2.6	74.5 ± 0.77	0.78			
	F	66 ± 2.9	71 ± 3.3	72 ± 2.2				
NDFap	IM	42 ± 5.8	53 ± 3.7	65 ± 2.2		0.023	<0.001	0.023
	CM	50 ± 7.3	62 ± 2.6	63 ± 1.6	1.41			
	F	49 ± 3.5	61 ± 5.3	61 ± 2.8				
TC	IM	617 ± 2.6	66 ± 3.0	74 ± 1.6		0.032	<0.001	0.042
	CM	66 ± 4.7	72 ± 2.8	72.6 ± 0.93	0.81			
	F	66 ± 2.7	71 ± 3.6	72 ± 2.6				

s.d., standard deviation; s.e.m, standard error of the mean; OM, organic matter; NDFap, neutral detergent fibre corrected for ash and protein; TC, total carbohydrates.
[a]Sex class: IM, intact males; CM, castrated males; F, females.
[b]Feed restriction: *ad libitum* (AL) or 300 or 600 g/kg/DM reduction of the AL intake.

diets are observed feed restriction, which can be explained by the reduction of the contribution of endogenous losses. The use of nitrogen compounds in the restricted animals was marked by decreased excretion of urinary nitrogen, indicated by a positive nitrogen balance, because there was intensified nitrogen recycling and increased conservation of compounds, reducing the amount of protein lost to the environment. Intact males had higher intake and nutrient utilization efficiency than either of the other classes. This is because males have higher energy and protein requirements for growth (Costa *et al*., 2013; Pereira *et al*., 2014), demanding higher intakes (NRC 2000, 2007).

Microbial nitrogen compounds were affected by feed restriction. Knowledge of microbial protein production (Fujihara *et al*., 2007) is indispensable for feed strategies, mainly to meet the protein requirements of animals (Valadares Filho *et al*., 2016). Thus, the use of PD excretion in urine is a useful tool to measure the production of nucleic acids by rumen microorganisms (Chen *et al*., 1990), and in feed-restricted animals, PD excretion measurement enables knowledge of the amino acids available in the duodenum (Chizzotti *et al*., 2008) so that the protein requirements for maintenance can be met (NRC 2001). There is little information on the excretion of PD in hair sheep. Creatinine excretion was constant, regardless of sex class and level of feed restriction. Creatinine is formed in a non-enzymatic and irreversible process of removing water from phosphocreatine in the muscle tissue (Harper *et al*., 1982).

Owing to its constant production and proportionality to the BW of an animal, creatinine is used to estimate the urinary excretion of ruminant animals in individual samples (David *et al*., 2015). Sheep subjected to dietary restriction decrease their DM intake and, consequently, there is a decreased flow of microbial nucleic acids into their intestines. This change is evidenced by reduced excretion of total PD, uric acid and allantoin, the largest single component of PD excreted in the urine. The excretion of allantoin for each sex class was 0.84, reflecting the complete excretion of PD, which was found to occur in a higher proportion of approximately 0.85 (Verbic *et al*., 1990). Hypoxanthine, xanthine and uric acid are excreted through urine with a high renal clearance rate constant of approximately 0.33/h in sheep (Chen *et al*., 1991) and cattle (Giesecke *et al*., 1993). In ruminant animals, most of the purine flowing into the small intestine is of microbial origin and absorbed exogenous purines are excreted into urine after the salvage pathways reach saturation. Excretion and CPmic production are proportional to DM intake, having strong correlations with the flow of microbial nitrogen compounds into the duodenum. However, the decreased rate of passage is attributed to low feed intake, which causes an increase in the use of nutrients, as demonstrated by increased nutrient digestibility.

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Table 5. Effects of sex class and feed restriction on nitrogen balance and purine derivatives in hair lambs (means and ± S.D.)

	Sex class (*S*)[a]			Feed restriction (FR)[b]				*P*-value		
Item	IM	CM	*F*	AL	300 g/kg/DM	600 g/kg/DM	S.E.M	*S*	FR	*S* × FR
Nitrogen, g/day[§]										
Intake	18 ± 6.1	16 ± 5.6	14 ± 4.7	22 ± 3.4	17 ± 1.8	10 ± 1.0	0.93	<0.001	<0.001	0.343
Faecal	6 ± 2.5	5 ± 2.0	3.9 ± 1.7	7 ± 1.6	5 ± 1.1	2.4 ± 0.33	0.36	<0.001	<0.001	0.061
Urinary	4 ± 1.1	5 ± 1.4	3.8 ± 0.71	5 ± 1.3	4.5 ± 0.70	3.2 ± 0.54	0.19	0.090	0.001	0.512
Retained	4.81 ± 2.5	4 ± 2.6	3 ± 2.3	7 ± 1.6	4.3 ± 0.97	1.2 ± 0.80	0.41	0.011	<0.001	0.534
Nitrogen balance	8 ± 2.8	7 ± 2.9	6 ± 2.6	10 ± 1.8	8 ± 2.0	4.0 ± 0.91	0.47	0.003	<0.001	0.423
BEN	3.3 ± 0.41	3.1 ± 0.40	3.0 ± 0.34	3.5 ± 0.30	3.3 ± 0.18	2.7 ± 0.16	0.07	<0.001	<0.001	0.372
EUL	6.2 ± 0.46	6.0 ± 0.43	5.6 ± 0.36	6.4 ± 0.35	6.1 ± 0.20	5.5 ± 0.17	0.07	<0.001	<0.001	0.313
Urine volume										
l/day	0.6 ± 0.18	0.8 ± 0.41	0.6 ± 0.27	0.5 ± 0.11	0.7 ± 0.24	0.7 ± 0.44	0.05	0.302	0.213	0.423
ml/day	565 ± 184.2	748 ± 410.1	596 ± 269.0	507 ± 111.2	678 ± 236.2	723 ± 443.1	51.9	0.303	0.213	0.423
Creatinine excretion										
mmol/day	0.02 ± 0.004	0.02 ± 0.005	0.02 ± 0.007	0.02 ± 0.004	0.02 ± 0.004	0.02 ± 0.008	0.001	0.702	0.853	0.882
mmol/kg BW/day	0.5 ± 0.13	0.4 ± 0.16	0.5 ± 0.20	0.5 ± 0.11	0.4 ± 0.12	0.5 ± 0.23	0.03	0.451	0.365	0.622
mmol/kg $BW^{0.75}$/day	1.0 ± 0.25	0.8 ± 0.33	1.0 ± 0.40	1.1 ± 0.22	0.8 ± 0.25	0.9 ± 0.46	0.06	0.465	0.234	0.611
Excretion of PD, mmol/day¶										
Allantoin	4 ± 1.4	4 ± 1.6	3.3 ± 0.92	5 ± 1.2	3.8 ± 0.86	2.5 ± 0.41	0.22	0.100	<0.001	0.402
Uric acid	0.4 ± 0.11	0.3 ± 0.13	0.3 ± 0.07	0.4 ± 0.13	0.3 ± 0.08	0.2 ± 0.04	0.02	0.007	<0.001	0.365
Xant and Hypoxant	0.6 ± 0.20	0.8 ± 0.43	0.7 ± 0.28	0.6 ± 0.12	0.8 ± 0.25	0.8 ± 0.48	0.06	0.313	0.295	0.401
AbsPD	5 ± 1.6	5 ± 1.8	4 ± 1.0	5 ± 1.4	5 ± 1.1	3.4 ± 0.89	0.19	0.092	<0.001	0.505
Total PD										
mmol/day	5 ± 1.6	5 ± 1.7	4.2 ± 0.94	6 ± 1.4	4.9 ± 0.99	3.5 ± 0.83	0.24	0.114	<0.001	0.485
mmol/$BW^{0.75}$	0.5 ± 0.12	0.6 ± 0.17	0.5 ± 0.09	0.6 ± 0.13	0.6 ± 0.11	0.5 ± 0.13	0.02	0.335	0.035	0.413
CPmic[††]										
g CP/day	21 ± 7.4	21 ± 8.1	18 ± 4.5	24 ± 6.5	21 ± 4.8	15 ± 4.1	0.85	0.092	<0.001	0.534
MPS										
g/kg de TDN	50 ± 8.3	55 ± 17.8	55 ± 11.9	47 ± 9.0	49 ± 8.8	62 ± 18.4	1.93	0.364	<0.001	0.152

S.D., standard deviation; S.E.M, standard error of the mean.
BEN, basal endogenous nitrogen loss; EUL, endogenous urinary losses; BW, body weight.
Total PD, total purine derivatives; Xant and Hypoxant, xanthine and hypoxanthine; CP, crude protein; MP, microbial protein synthesis; TDN, total digestible nutrients; AbsPD, purine derivative absorption.
[a]Sex class: IM, intact males; CM, castrated males; F, females.
[b]Feed restriction: *ad libitum* (AL) or 300 or 600 g/kg/DM reduction of the AL intake.
[§]Nitrogen, amounts of Nitrogen
[¶]Excretion of DP, Excretion of Digestible Protein
[††]CPmic, microbial crude protein

Table 6. Infrared thermography (°C), rectal temperature (°C) and heat tolerance coefficient according to sex class, feed restriction and time period in hair lambs (means and ± s.d.)

Item	Sex class (S)[a]			Feed restriction (FR)[b]			Period (P)[c]			P-value						
	IM	CM	F	AL	300 g/kg	600 g/kg	M	A	s.e.m	S	FR	P	$S \times$ FR	$S \times P$	FR $\times P$	$S \times$ FR $\times P$
Infrared thermography																
Right side	34.4 ± 0.64	34.4 ± 0.66	34.2 ± 0.80	34.5 ± 0.67	34.6 ± 0.63	34.0 ± 0.74	33.8 ± 0.43	34.9 ± 0.34	0.084	0.010	<0.001	<0.001	0.041	0.500	0.375	0.882
Left side	34.5 ± 0.70	34.5 ± 0.69	34.3 ± 0.75	34.6 ± 0.67	34.6 ± 0.61	34.1 ± 0.74	33.8 ± 0.47	35.0 ± 0.32	0.085	0.092	<0.001	<0.001	0.091	0.962	0.165	0.623
Pectoral region (anterior aspect)	34.4 ± 0.68	34.6 ± 0.59	34.4 ± 0.66	34.6 ± 0.65	34.5 ± 0.65	34.3 ± 0.66	33.9 ± 0.30	35.0 ± 0.29	0.076	0.071	0.005	<0.001	0.172	0.751	0.833	0.232
Rectal temperature																
	40.2 ± 0.16	40.1 ± 0.25	40.1 ± 0.29	40.3 ± 0.16	40.2 ± 0.21	39.8 ± 0.20	40.1 ± 0.30	40.2 ± 0.26	0.034	0.162	<0.001	0.013	0.050	0.971	0.422	0.913
Heat tolerance coefficient																
	81 ± 5.8	82 ± 4.4	82.9 ± 5.18	78 ± 2.8	80 ± 3.7	87 ± 3.1	–	–	0.852	0.293	<0.001	–	0.511	–	–	–

s.d., standard deviation; s.e.m, standard error of the mean.
[a]Sex class: IM, intact males; CM, castrated males; F, females.
[b]Feed restriction: *ad libitum* (AL) or 300 or 600 g/kg/DM reduction of the AL intake.
[c]Period: M, morning; A, afternoon.

Table 7. Infrared thermography according to different sex classes and feed restriction levels in hair lambs during different time periods (means and ± s.d.)

		Morning			Afternoon										
		Feed restriction (FR)[b]			Feed restriction				P-value						
Item	Sex class (S)[a]	AL	300 g/kg/DM	600 g/kg/DM	AL	30%	60%	s.e.m	S	FR	P[c]	$S \times$ FR	$S \times P$	FR $\times P$	$S \times$ FR $\times P$
IRT right side (°C)[d]	IM	34.1 ± 0.62	33.9 ± 0.12	33.7 ± 0.20	34.9 ± 0.28	35.0 ± 0.19	35.0 ± 0.34	0.084	0.011	<0.001	<0.001	0.042	0.502	0.375	0.881
	CM	34.0 ± 0.20	34.2 ± 0.36	33.5 ± 0.33	35.0 ± 0.12	35.3 ± 0.23	34.7 ± 0.08								
	F	33.7 ± 0.53	33.9 ± 0.13	33.0 ± 0.20	35.1 ± 0.34	35.1 ± 0.41	34.4 ± 0.28								

s.d., standard deviation; s.e.m, standard error of the mean.
[a]Sex: IM, intact males; CM, castrated males; F, females.
[b]Feed restriction: *ad libitum* (AL) or 300 or 600 g/kg/DM levels of the AL intake.
[c]Period: M, morning; A, afternoon.
[d]IRT right side = infrared thermography of the right side.

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Thus, minor losses of nitrogen and maximizing synchronization between dietary protein and carbohydrates in the rumen can result in improved efficiency of the use of microbial protein, as can be observed in animals subjected to 600 g/kg DM feed restriction. Microbial efficiency can be represented by the production of microbial cells (number or weight) synthesized by the substrate unit used. For the nutritional requirements of ruminant systems, microbial efficiency can be expressed as a function of TDN (NRC 2001, 2007), fermentable metabolizable energy (AFRC 1993) or the availability of carbohydrates in the rumen.

In tropical or semi-arid conditions, fibrous carbohydrates are the main source of energy in the rumen, and the grasses of these regions have average or low levels of protein; the availability of *N* in the rumen may be the main factor limiting microbial growth in this compartment. The average MPS was estimated at 53.1 g/kg of TDN, but less than the mean value of 76.2 g CPmic per kg of TDN was obtained in studies with sheep and goats in tropical regions receiving control diets (Fonseca *et al.*, 2006; Carvalho *et al.*, 2010; Pereira *et al.*, 2016).

Animals undergoing long periods of food shortage alter their physiological processes, becoming more adapted to the hot climate, changing their typical ingestive behaviour and the use of ingested nutrients while decreasing their energy expenditure for maintenance to ensure thermoregulation. Moreover, in high-temperature environments, productivity is compromised, given that feed intake is reduced and is usually correlated positively with heat production in fasting. Thus, animals that are acclimated to heat alter their physiological processes in order to reduce heat production (Starling *et al.*, 2005). The surface temperatures found in the current study demonstrated that dietary restriction minimizes heat loss through the skin of the animal. Obviously, this result occurred because the animals maximized the use of nutrients in their bodies, generating less body heat through metabolic processes (Kleiber, 1975). The physiological parameters of ruminants are directly influenced by climatic factors such as temperature, RH and solar radiation (Furtado *et al.*, 2012). Typically, an increase in the ambient temperature is accompanied by an increase in the RT of the animal, requiring additional effort to ensure thermoregulation. Despite the differences found in the current study, the RT values remained within the range of physiological values suggested by Marek and Mócsy (1973) in young animals up to 1 year old (38.5–40.5 °C). These results demonstrated the adaptability of hair sheep to the tropical climate even under critical stress conditions in the afternoon, as demonstrated by the THI (80.48). Males and females at 600 g/kg/DM feed restriction had decreased values for body temperature on the right side. However, for the left side and the front aspect of the chest, a similar finding was recorded in all animals restricted to 600 g/kg/DM feed. The lower feed intake resulted in less time spent on feeding behaviour and rumination, reducing the number of calories expended through those activities. According to Lachica and Aguilera (2005) and Cannas *et al.* (2003), the time spent on feeding activities can increase animal heat production by 10–26%, resulting in higher energy costs. The variation in body temperature was greatest on the left side of the animals owing to the topographic location of the stomachs, which, according to Ørskov and Ryle (1990), make this region of the body a window to the temperature variations within.

Infrared thermography (IRT) is an important non-invasive tool that measures the surface temperature of an animal and identifies many physiological and pathological processes related to changes in body temperature (Montanholi *et al.*, 2008; Church *et al.*, 2014). Another factor that influences the variation of body surface temperature is the environmental temperature, which affects the skin surface temperature and heat exchange between the organism and environment (Collier *et al.*, 2006). The animals with the lowest surface temperatures on the left and right sides were those subjected to 600 g/kg/DM feed restriction. These results show that the feed restriction minimizes heat loss through the animal's skin. Less heat loss occurs because the animal maximizes the use of nutrients by the body, causing less heat to be released during endogenous reactions (Kleiber, 1975). In addition, lower feed intake resulted in less time spent on feeding and rumination activities, reducing the expenditure of calories on ingestive behaviour. According to McManus *et al.* (2016), animals that have lower body surface temperatures are more efficient animals.

Conclusion

Feed restriction reduces the time spent on feeding and rumination but on the other hand improves the digestibility of nutrients, balance of nitrogen compounds and the 600 g/kg/DM level maximizes the efficiency of microbial synthesis, while the gender factor influence only the NB. In addition, IRT indicated that males and females subjected to 600 g/kg/DM feed restriction have increased heat tolerance coefficients.

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Conflict of interest. None.

Ethical standards. The current study was conducted at the Department of Animal Science at the Federal University of Ceara in strict accordance with the recommendations of the Guide for the Care and Use of Agricultural Animals in Research and Teaching and was approved by the Committee on the Ethics of Animal Experiments of the Federal University of Ceara, Ceara State, Brazil. This research was submitted to and approved by the Research Ethics Committee on Animals (CEUA) according to its guidelines (UFC – protocol number 98/2015).

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