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High-Concentrate Diets Improve Performance but Challenge Thermoregulation in Sheep

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ABSTRACT

High-concentrate diets improve body performance and alter diet digestion rates, potentially affecting thermoregulation and well-being in sheep. This study aimed to evaluate the effect of increasing concentrate on intake, digestibility of nutrients, ingestive behavior, and physiological parameters in sheep. Twenty-four male Dorper lambs with an initial weight of 16.8 ± 3.5 kg and an age of 60 ± 4.5 days were randomly assigned to diets with increasing levels of concentrate: 45%, 59%, 73%, and 87%. Tifton 85 hay was used as a fiber source. The data were analyzed as a completely randomized design and subjected to orthogonal contrast analysis for linear and quadratic effects for dietary concentrate levels as a fixed effect, at a significance level of p≤0.05. The increase in concentrate increased daily weight gain (0.094 to 0.233 kg/day), dry matter intake, number of meals, and physiological parameters, while reducing neutral detergent fiber intake, digestibility of nutrients, rumination time, and meal duration. The heart rate, respiratory rate, and rectal temperature were positively correlated with concentrate level and negatively correlated with meal duration and total chewing time. Increasing concentrate improves the intake of nutrients and the performance of lambs, but it also increases thermoregulatory rates. Diets with 73% concentrate allow for higher performance with a slight increase in rectal temperature. Physiological parameters are more affected by concentrate levels than by fiber levels.

***Keywords:** carbohydrate; neutral detergent fiber; respiratory rate; temperature stress; thermoregulation*

INTRODUCTION

Modern sheep meat production systems have focused on slaughtering young animals to ensure product quality. The accelerated development of muscle and fat tissues in animals, along with increased weight gain in a shorter period, is possible with high levels of grains in diets (Wang *et al*., 2024), which can also lead to a reduction in rumen pH, affecting animal performance and health.

The effect of diets on animal health, particularly in high-concentrate diets, should be considered beyond just digestive aspects to ensure the well-being of the animals (Kotsampasi *et al*., 2024). In tropical regions, the diet influences the susceptibility of animals to environmental effects (Conte *et al*., 2018), as the endogenous heat produced during food metabolism intensifies the thermal effects of the environment on their thermoregulatory physiology. Therefore, the interaction between climate and nutrition needs to be considered when evaluating the productive parameters and welfare of animals in production systems.

Some studies carried out diverge in describing fiber (Talmón *et al*., 2023) or grains (Nobre *et al*., 2016; Pereira *et al*., 2022) as the main contributors to the production of metabolic heat. Evaluating the effect of diets with a high (70%) or low (30%) concentrate proportion, Pereira *et al*. (2022) described that high-concentrate diets negatively affected the thermoregulation of confined sheep, with an increase in respiratory rate and rectal temperature. These thermoregulatory pathways for dissipating latent heat result in energy expenditure and can reduce feeding activities, thereby decreasing the availability of nutrients for the weight gain of sheep (Santos *et al*., 2019).

Previous studies (Pereira *et al*., 2022) focused solely on comparing low- and high-concentrate diets, restricting the interpretation of sheep production systems to scenarios that either maximize body performance or prioritize animal well-being. However, intermediate concentrate levels are commonly adopted across different production systems and stages in sheep farming, and may represent viable alternatives for enhancing productivity without impairing thermoregulatory physiology. Evaluating different concentrate levels in our study provides a more comprehensive understanding of diets that support both environmental and nutritional well-being, without compromising production. This approach also helps producers define nutritional strategies and determine safe limits for the inclusion of grain in sheep diets.

Based on this rationale, we hypothesized that diets containing approximately 59% concentrate would promote a balance between achieving favorable growth rates and preserving physiological function and animal welfare. Therefore, we aimed to evaluate the body performance, nutritional, behavioral, and thermoregulatory parameters of lambs fed different concentrate levels in a tropical region.

MATERIALS AND METHODS

Location of the Study and Ethical Statement

The animal study protocol was approved by the Animal Ethics Committee of Universidade Federal do Rio Grande do Norte (UFRN/Brazil), 050.057/2017, approved on October 10, 2017. The experiment was conducted at Macaíba, RN, Brazil (5°51′36′′ S latitude and 35°20′59′′ W longitude). The region's climate is Aw, with an average annual temperature of 25.8 ºC and a surplus in rainfall from May to August. The air temperature (AT, ºC) and relative humidity (RH, %) were collected using a thermohygrometer (Figure 1). The Temperature and Humidity Index (THI) was determined according to Mader *et al*. (2006):

$$THI = 0.8 \times AT + ((RH/100) \times (AT - 14.3)) + 46.4$$

Animals, Diets, and Experimental Design

Twenty-four male Dorper lambs, with an average initial body weight of 16.8 ± 3.5 kg and an average age of 60 ± 4.5 days, were confined in individual covered pens (1.0 x 1.0 m) provided with feeders and drinkers, and a concrete floor. The experiment lasted for 85 days, with the first 15 days used for the animals to adapt to the diets, facilities, and management.

The animals were arranged in a completely randomized design, and fed diets with different concentrate levels: 45%, 56%, 73%, and 87% (Table 1). The concentrate levels were selected to represent a biologically and practically relevant gradient from moderate to very high concentrate inclusion commonly used in sheep production and finishing systems (Parente, 2016; Jin & Zhou, 2021; Arjmand *et al*., 2022; Lima *et al*., 2024). The diets were formulated to be isoproteic, but the energy content increased with increasing concentrate. The TDN (total digestible nutrients) was determined by BR-CAPRINOS & OVINOS (2024): TDN, % = EEd × 2.25 + CNFd + CPd + FDNd – 4. where, EEd = digestible ether extract; CNFd = digestible non-fibrous carbohydrates; CPd = digestible crude protein and FDNd = digestible neutral detergent fiber. The composition of foods and diets is described in Table 1.

The diets were offered as a total mixed ration consisting of Tifton 85 hay (*Cynodon* spp.) ground in an agricultural silage maker into particles of 1.5 cm and standard concentrate. Food was offered once a day (07:00), and the amount was calculated based on the previous day's intake, ensuring ad libitum intake by the animals, with a daily adjustment of 10% of leftovers on a dry matter (DM) basis.

Data and Sample Collection

Animals were weighed at the beginning and end of the experiment, before feeding. The nutrient digestibility coefficient was determined by estimating fecal production using spot collections of feces taken directly from the animals' rectums on different days and at different times (08:00, 10:00, 12:00, 14:00, and 16:00) (Almeida *et al*., 2018), between the 35th and 40th day of the experimental period. During this period, samples of feed and leftovers were also collected daily to determine nutrient intake.

Feeds, leftovers and feces were pre-dried and ground in a grinding mill with 1 mm and 2 mm sieves to determine of dry matter (DM) mineral matter (MM), organic mat-ter (OM), crude protein (CP), ether extract

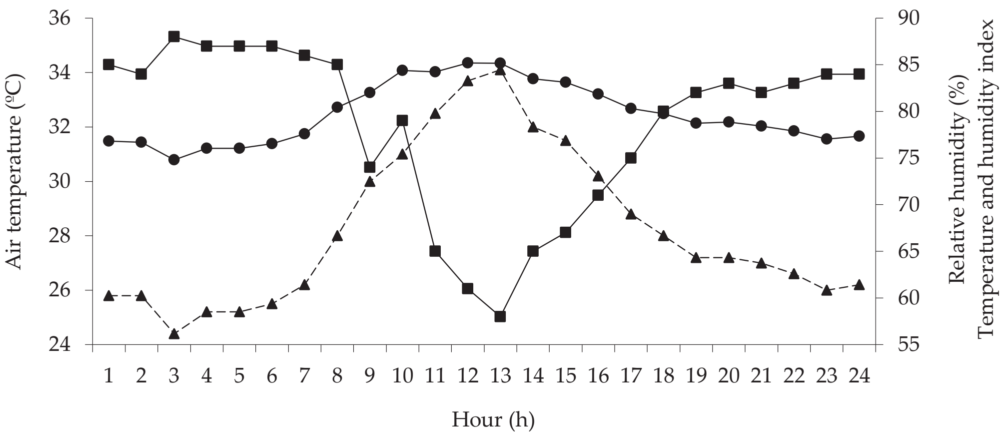

Figure 1. Air temperature (ºC), relative humidity (%), and temperature and humidity index (THI) during the experiment. Average of environmental assessments (n = 4) during a 24-hour interval, during evaluation of physiologic parameters. Note: --▲-- = AT (ºc); —■— = RU (%); —●— = THI.

Table 1. Chemical composition of feed and experimental diets with different levels of concentrate for sheep

Nutritional attributes	Feeds			Concentrate levels (%)			
	TH	CF	SM	45	59	73	87
Ingredients							
Tifton 85 hay	-	-	-	55.0	41.0	27.0	13.0
Corn flour	-	-	-	25.0	39.0	53.0	66.0
Soybean meal	-	-	-	16.0	16.0	16.0	17.0
Mineral salt	-	-	-	4.0	4.0	4.0	4.0
Nutrient composition (%)							
Dry matter[1]	89.55	89.35	88.59	89.72	89.70	89.67	89.63
Organic matter[2]	90.94	98.07	93.68	89.52	90.52	91.52	92.47
Crude protein[2]	9.16	9.12	44.82	14.49	14.48	14.48	14.83
Ether extract[2]	2.21	4.69	1.55	2.64	2.98	3.33	3.65
Mineral matter[2]	9.06	1.93	6.32	10.44	9.44	8.44	7.49
Neutral detergent fiber[2]	72.56	7.81	22.18	45.41	36.34	27.28	18.36
Acid detergent fiber[2]	41.80	4.88	13.89	26.43	21.26	16.09	11.02
TDN	-	-	-	38.50	54.56	59.18	60.09

Note: TH = Tifton 85 hay (*Cynodon* spp.); CF = Corn flour; SM = Soybean meal; TDN = total digestive nutrient. [1]As fed percentage; [2]Dry matter percentage.

(EE), neutral detergent fiber (NDF) and acid detergent fiber (ADF) following (Detmann *et al*., 2021). Non-fiber carbohydrates (NFC) according to Mertens (1996).

The samples processed in 2 mm were placed in non-woven bags and incubated in the rumen of cannulated sheep for 288 hours (Valente *et al*., 2015). After incubation, the residual material was subjected to extraction with neutral detergent to determine the indigestible neutral detergent fiber (iNDF; Valente *et al*., 2015), used as an internal marker to estimate the animals' fecal production. Nutrient intake was determined by the difference between the amount of nutrients in the diet and leftovers. Digestibility was determined by the ratio of nutrient intake to fecal composition.

Ingestive behavior was assessed between the 41st and 42nd experimental days. The animals were subjected to visual observation every five minutes for 24 hours (Burguer *et al*., 2000) to determine the time spent feeding, ruminating, idling, and grooming, as well as their social interactions with other animals in adjacent stalls and/or evaluators. Total chewing time (TCT) was obtained from feeding and rumination time (Burguer *et al*., 2000). During the nocturnal observations, the environment was kept under artificial lighting.

Mastication dynamics were studied by counting rumen boluses and measuring the time taken to chew them during four evaluation periods: 09:00 to 12:00, 15:00 to 18:00, 21:00 to 00:00, and 03:00 to 06:00. The number of boluses and chews determined the chewing rate counted over 24 hours (Burguer *et al*., 2000). Feeding and rumination efficiencies, as a function of dry matter (DM) and neutral detergent fiber (NDF), were obtained from the ratio between nutrient intake and time feeding and ruminating (Burguer *et al*., 2000).

The feeding pattern was defined based on the reasoning (Mullins *et al*., 2012) for a 12-minute interval. One feeding was defined by at least two consecutive 5-minute feed intake events after at least two consecutive 5-minute idleness or rumination events. The average feeding duration was obtained from the ratio between feeding time (min/d) and the number of feedings per day. The duration of the first feeding was obtained by considering the animals' first feeding after being offered feed. The duration of the longest feeding during the entire behavioral assessment was determined. Feeding size was obtained from the ratio between daily dry matter intake (DMI, kg/d) and the number of feedings/d.

Animal physiological parameters were measured on the 14th, 28th, 42nd, and 56th experimental days at 09:00 and 15:00. Respiratory rate (RR; movements per minute - mpm) was measured by counting the lateral movements of the animal's flanks, while heart rate (HR; beats per minute - bpm) was obtained from the number of heartbeats. Both assessments were conducted using a stethoscope for 15 uninterrupted seconds, and the results were then converted to a 60-second scale. To measure rectal temperature (RT, ºC), a digital clinical thermometer was inserted into the animals' rectal ampulla, and the bulb remained close to the mucosa until it stabilized. The average between four evaluations was taken for each parameter assessed.

Statistical Analysis

The data were analyzed using mixed effects analysis procedures (PROC MIXED) of the SAS (SAS University Edition, Cary, NC), considering a completely randomized design, submitted to residual normality (Shapiro-Wilk) and homogeneity of variances (Levene) tests ($p\leq0.05$). When necessary, data were transformed by the Box-Cox method and/or analyzed for heterogeneity of variance (SAS GROUP option). The data were subjected to an orthogonal contrast analysis of the linear and quadratic effects of concentrate levels, and the means were compared using the Tukey test at a significance level of $p\leq0.05$.

The physiological parameters were also subjected to the effects of time (9:00 a.m. and 3:00 p.m.) and interaction with the levels of concentrate in the diet, using the F-test at $p\leq0.05$. The nutritional, behavioral, and physiological parameters were subjected to Pearson's correlation analysis (PROC COR) with significance at p≤0.05.

Distribution (%) ingestive behavior (feeding time per hour relative to total daily observation time) was analyzed using PROC GLIMMIX, considering the fixed effects of treatment, hour, and their interaction. Animal nested within treatment was included as a random effect. The repeated structure over time was modeled using an autoregressive covariance matrix [AR (1)]. Least square means were compared using Tukey's adjustment, and significance was declared at p≤0.05.

RESULTS

Environmental Variables

During the experiment, the AT and THI increased starting at 8:00 a.m., remaining above 28 °C and 80 for AT and THI, respectively, until 6:00 p.m., with a decline from 7:00 p.m. THI remained below 80 between 7:00 p.m. and 7:00 a.m., with a minimum of 75 at 3:00 a.m.

Nutritional and Behavior Variables

The concentrate linearly increased final body weight (FW) and the average daily weight gain (ADG) throughout the study (p<0.01; Table 2). The ADG increased by 140 g/day from concentrate levels of 45% to 87%, resulting in an increase of approximately 43.5% in FW.

Except for neutral detergent fiber intake (NDFI; kg/day), the nutrient intake (kg/day) increased linearly with the increase in concentrate in the diet (p<0.01; Table 2). DMI increased by approximately 61.6% – equivalent to 0.282 kg/day – between levels 45% and 87% of concentrate. NDFI showed quadratic behavior with a maximum point of 0.251 kg/day in diets with 59% concentrate. DMI and NDFI in relation to body weight (%BW) showed increasing and decreasing linear responses, respectively, as concentrate increased in the diet (p<0.01).

The increase in concentrate linearly reduced the nutrient digestibility coefficient (p≤0.05; Table 2). The digestibility of NDF fell sharply (p<0.01) from 87.53% to 39.87% in diets with concentrate contents from 45 to 87%. The time spent on feeding (FET), rumination (RUT), and total chewing (TCT) decreased linearly with the increase in concentrate in the diet (p<0.01; Table 3), an opposite result to that recorded for the time spent idling (IDT; p<0.01). Grooming activities were not affected by the concentrate levels of diet (p<0.01).

Dry matter feeding and rumination efficiencies (dmFE and dmRE; kg DM/h) increased linearly with the increase in concentrate (p<0.01; Table 3), which means there was greater DM intake and rumination per unit of time in response to the increase in DM and reduction in FET and RUT. The increase in concentrate quadratically affected the NDF feeding (ndfFE) and rumination (ndfRE) efficiencies (p<0.05), with maximum points equivalent to 0.063 and 0.050 kg NDF/h for lambs fed diets with 73% and 59% of concentrate, respectively.

Considering the circadian cycle of the animals, regardless of the level of concentrate in the diet, the animals had a predominantly diurnal feeding habit with feeding peaks in the early morning, which includes

Table 2. Nutrient intake, body performance, and nutrient digestibility coefficient of confined sheep fed diets with increasing levels of concentrate

Assessment variables	Concentrate levels (%)				SEM	P value	
	45	59	73	87		L	Q
Body performance, kg							
Initial body weight	17.0	16.8	17.0	16.8	0.72	0.95	0.99
Final body weight	23.6[b]	29.4[a]	33.2[a]	33.1[a]	1.38	<0.01	0.19
Average daily weight gain	0.094[c]	0.180[b]	0.231[a]	0.233[a]	0.01	<0.01	0.06
Nutrient intake, kg/d							
Dry matter	0.458[b]	0.641[a]	0.737[a]	0.740[a]	0.03	<0.01	0.07
Organic matter	0.408[b]	0.577[a]	0.672[a]	0.683[a]	0.03	<0.01	0.08
Mineral matter	0.019[b]	0.030[a]	0.037[a]	0.032[a]	0.01	<0.01	0.01
Crude protein	0.068[c]	0.093[b]	0.110[ab]	0.116[a]	0.01	<0.01	0.15
Ether extract	0.015[c]	0.021[b]	0.027[a]	0.030[a]	0.01	<0.01	0.31
Neutral detergent fiber	0.228[a]	0.251[a]	0.209[a]	0.119[b]	0.01	<0.01	<0.01
Non-fiber carbohydrate	0.098[d]	0.220[c]	0.326[b]	0.418[a]	0.03	<0.01	0.51
Nutrient intake, %body weight							
Dry matter	2.27[b]	2.83[a]	2.93[a]	3.01[a]	0.10	<0.01	0.12
Neutral detergent fiber	1.14[a]	1.13[a]	0.82[b]	0.49[c]	0.01	<0.01	0.06
Nutrient digestibility, %							
Dry matter	88.0[a]	86.9[a]	79.5[b]	79.0[b]	1.64	0.01	0.92
Organic matter	88.8[a]	88.1[a]	80.9[b]	80.1[b]	1.55	0.01	0.98
Crude protein	89.3[a]	87.5[ab]	80.9[b]	82.8[b]	1.44	0.02	0.45
Neutral detergent fiber	87.5[a]	83.3[a]	66.7[b]	39.9[c]	4.50	<0.01	0.06

Note: SEM = standard error of the mean; L = linear contrast; Q = quadratic contrast. Means followed by different superscript letters differ (Tukey test, p<0.05).

the time of feeding management as a stimulating activity for the animals to ingest the feed. At the same time, rumination rose predominantly at night, more intensely between 03:00 and 06:00, possibly due to the lower temperatures (Figure 1), contrary to the behavior of the predominant idleness during the day, when temperatures are higher (Figure 2). The concentration level does not affect the circadian rhythm of the animals ($p<0.05$; Figure 2).

All parameters for evaluating rumination dynamics showed quadratic behavior with an increase in concentrate in the diet ($p<0.01$; Table 4). The number of chews per bolus (NCb, n/bolus) and the chewing rate (CR, n/s) were reduced in diets with 59% concentrate. The diet

Table 3. Behavioral activities, and feeding and rumination efficiencies (based on DM and NDF) of confined sheep fed diets with increasing levels of concentrate

Behavior variables	Concentrate levels (%)				SEM	P value	
	45	59	73	87		L	Q
Behavioral activities time, min/day							
Feeding	313.3[a]	287.5[a]	230.4[b]	182.5[c]	12.81	<0.01	0.44
Rumination	439.2[a]	340.0[b]	348.8[b]	306.7[b]	14.25	<0.01	0.16
Total chewing	752.5[a]	627.5[b]	579.2[b]	489.2[c]	23.57	<0.01	0.49
Idleness	599.6[c]	718.3[b]	770.8[b]	853.8[a]	22.21	<0.01	0.31
Grooming	87.9	94.2	90.0	97.0	5.97	0.13	0.39
Feeding and rumination efficiencies							
dmFE, kg DM/h	0.131[c]	0.191[b]	0.259[a]	0.280[a]	0.02	<0.01	0.36
dmRE, kg NDF/h	0.093[b]	0.161[a]	0.164[a]	0.170[a]	0.01	<0.01	0.06
ndfFE, kg DM/h	0.046[b]	0.060[a]	0.063[a]	0.043[b]	0.02	0.86	0.01
ndfRE, kg NDF/h	0.036[b]	0.050[a]	0.040[b]	0.026[c]	0.01	0.02	<0.01

Note: dmFE= dry matter feeding efficiency (kg DM/h); ndfFE= neutral detergent fiber feeding efficiency (kg NDF/h); dmRE = dry matter rumination efficiency (kg DM/h); ndfRE = neutral detergent fiber rumination efficiency (kg NDF/h); SEM = standard error of the mean; L = linear contrast; Q = quadratic contrast. Means followed by different superscript letters differ (Tukey test, $p<0.05$).

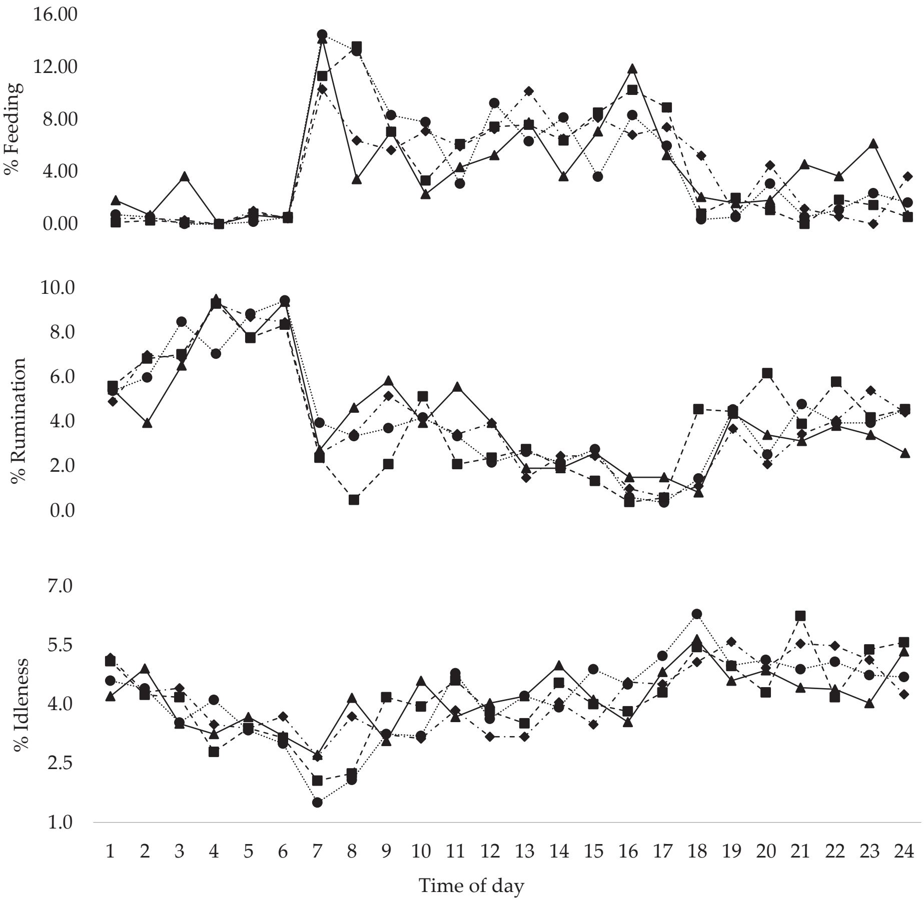

Figure 2. Distribution (%) over 24 24-hour period of feeding, rumination, and idleness time of confined sheep fed diets with increasing levels of concentrate. There was no effect ($p>0.05$) of concentrate levels on the distribution of ingestive activities throughout the evaluation day. CONC = concentrate level in the diets (45%, 59%, 73%, and 87%). Note: --■-- = 45% CON; -·◆·- = 59% CON; ·····●····· = 73% CON; —▲— = 87% CON.

with 73% concentrate resulted in a maximum chewing time of 49.3 seconds per rumen bolus (CTb, s/bolus), but reduced the total daily number of chews (NCd, 34,147 n/day) and ruminal boluses (NBd, 437.2 n/day).

The number of feedings (NF) and size feeding (SF, kg DM/feeding) increased linearly, while the duration (DF), duration of the first (DFF), and longest (DLF) feedings decreased linearly as the concentrate in the diets increased (p<0.01; Table 4).

Physiological Variables

Respiratory rate (RR), heart rate (HR), and rectal temperature (RT) increased linearly (p<0.05) due to the concentrate increase in the diets in both shifts (Table 5). Considering only the evaluation shifts alone, RR and RT were significantly higher (p<0.01) in the afternoon (15:00), recording 110.9 mpm vs. 91.4 mpm and 39.7 ºC vs. 39.5 ºC when compared to the morning. There was no effect (p=0.34) of evaluation time on HR (104.9 vs 111.3 beats/min at 09:00 and 15:00, respectively). There was also no effect of interaction between the concentrate levels and the evaluation times on the physiological parameters (p>0.05; Table 5).

The increase in the level of concentrate in the roughage:concentrate ratio of the diet showed positive correlation with OMI, IDT, NCb, and NF, and negative correlation with OMD, TCT, and DF. NF correlated negatively with OMD and TCT, and positively with IDT, while DF correlated negatively with OMI, IDT, and NF, and positively with TCT. The SF correlated moderately and negatively with OMI (Table 6).

The physiological parameters showed positive and strong effects (RT and HR) and moderate effects (RR) with the increase in the level of concentrate and the reduction of roughage. RT, HR, and RR correlated negatively with TCT and DF and positively with IDT. RT and RR correlated moderately positively with NCb, and HR correlated moderately positively with OMI and negatively with OMD.

DISCUSSION

Environmental Variables

THI was used to assess the animals' comfort and thermal stress, according to (McManus *et al*., 2016; Slimen *et al*., 2019; Ferreira *et al*., 2024): <72 (comfortable), 72–77 (moderate stress), 78–89 (severe stress), and from 90 (extreme stress). The animals remained under heat stress throughout the study, with moderate stress from 11:00 p.m. to 6:00 a.m., and severe stress from 7:00

Table 4. Rumination and feeding dynamics of confined sheep fed diets with increasing levels of concentrate

Behavior variables	Concentrate levels (%)				SEM	P value	
	45	59	73	87		L	Q
Chewing dynamics							
NCb, n/bolus	80.5^{b}	69.8^{d}	78.3^{c}	86.5^{a}	1.27	<0.01	<0.01
CTb, s/bolus	41.1^{b}	48.3^{a}	49.3^{a}	40.7^{b}	0.85	0.99	<0.01
NCd, n/day	60,086^{a}	35,686^{c}	34,147^{d}	40,434^{b}	21.61	<0.01	<0.01
NBd, n/day	748.1^{a}	511.3^{b}	437.2^{d}	469.5^{c}	25.47	<0.01	<0.01
CR, n/s	2.0^{b}	1.5^{d}	1.6^{c}	2.1^{a}	0.06	<0.01	<0.01
Feeding dynamics							
NF, n/day	9.2^{c}	9.8^{b}	10.8^{a}	10.8^{a}	0.22	<0.01	0.38
DF, min	39.0^{a}	30.8^{a}	22.4^{b}	17.2^{b}	2.15	<0.01	0.56
DFF, min	81.0^{a}	71.7^{a}	54.2^{b}	50.8^{b}	3.79	<0.01	0.59
DLF, min	85.0^{a}	79.2^{a}	59.2^{b}	57.5^{b}	3.64	<0.01	0.69
SF, kg DM/feeding	0.052^{b}	0.066^{a}	0.068^{a}	0.069^{a}	0.01	0.03	0.21

Note: NCb = number of chews per bolus (n/bolus); CTb = chewing time per bolus (s/bolus); NCd = number of chews per day (n/day); NBd = number of boluses per day (n/day); CR = chewing rate (n/s); NF = number of feedings (n/day); DF = duration of feedings (min); DFF = duration of first feeding (min); DLF = duration of longest feeding (min); SF = size of feeding (kg DM/feeding); SEM = standard error of the mean; L = linear contrast; Q = quadratic contrast. Means followed by different superscript letters differ (Tukey test, p<0.05).

Table 5. Physiological variables of confined sheep fed diets with increasing levels of concentrate

Time	Concentrate levels (%)				SEM	P value			
	45	59	73	87		L	Q	T	C*T
Respiratory rate (mov/min[1])									
9:00 a.m.	79.3^{b}	88.7^{b}	90.3^{b}	107.3^{a}	3.33	<0.01	0.46	<0.01	0.94
3:00 p.m.	102.0^{b}	104.5^{b}	109.2^{b}	127.8^{a}	3.69	<0.01	0.21		
Heart rate (beat/min[1])									
9:00 a.m.	90.2^{d}	100.8^{c}	108.2^{b}	120.4^{a}	2.68	<0.01	0.78	0.34	0.22
3:00 p.m.	102.0^{b}	105.8^{b}	115.2^{a}	122.0^{a}	2.27	<0.01	0.63		
Rectal temperature (ºC)									
9:00 a.m.	39.3^{c}	39.4^{b}	39.4^{b}	39.8^{a}	0.08	0.01	0.31	<0.01	0.90
3:00 p.m.	39.5^{b}	39.6^{b}	39.6^{b}	40.0^{a}	0.07	<0.01	0.26		

Note: T = time (9:00 a.m. or 3:00 p.m.); C = levels of concentrate (45%, 59%, 73%, or 87%). Means followed by different superscript letters differ (Tukey test, p<0.05).

Table 6. Pearson's correlation coefficients between nutrient intake, feeding activities, and physiological variables of confined sheep fed diets with increasing levels of concentrate

	CONC	OMI	OMD	TCT	IDT	NCb	CTb	NF	DF	SF	RT	HR	RR
CONC	1.00	0.65**	-0.54*	-0.83**	0.80**	0.49*	0.32	0.66**	-0.80**	0.39	0.63**	0.86**	0.51*
OMI		1.00	-0.33	-0.55*	0.48*	0.18	0.21	0.38	-0.62**	0.86**	0.33	0.53*	0.32
OMD			1.00	0.58*	-0.63**	-0.08	-0.08	-0.46*	0.37	-0.20	-0.28	-0.51*	-0.42
TCT				1.00	-0.97**	-0.27	-0.10	-0.54**	0.74**	-0.31	-0.41*	-0.60**	-0.36
IDT					1.00	0.24	0.15	0.59**	-0.70**	0.22	0.43*	0.59**	0.39
NCb						1.00	-0.70**	0.18	-0.33	0.05	0.45*	0.30	0.46*
CTb							1.00	0.22	-0.07	0.21	-0.25	-0.15	-0.05
NF								1.00	-0.81**	0.02	0.29	0.32	0.22
DF									1.00	-0.24	-0.44*	-0.69**	-0.45*
SF										1.00	0.19	0.22	0.41
RT											1.00	0.68**	0.57**
HR												1.00	0.74**
RR													1.00

Note: CONC = concentrate level in the diets (45%, 59%, 73%, and 87%); OMI = Organic matter intake (kg/d); OMD = organic matter digestibility (%); TCT = Total chewing time (min/d); IDT = Idleness time (min/d); NCb = number of chews per bolus (n/bolo); CTb = chewing time per bolus (s/bolo); NF = Number of feedings (n/day); DF = Duration of feedings (min/n); SF = size of feeding (kg/day); RT = Rectal temperature (ºC); HR = Heart rate (beat/min1); RR = Respiratory rate (mov/min1). *0.01 ≥ p ≤ 0.05 and **p<0.01

a.m. to 10:00 p.m. (Figure 1). The period between 10:00 a.m. and 2:00 p.m. was more critical for animals, with a THI between 84 and 85, while the period between 3:00 and 5:00 a.m. showed a lower THI, between 75 and 76.

Nutritional and Behavior Variables

The proportions of grains and fiber in the diet influence voluntary feed intake in ruminants. In our study, the increase in fiber and low-concentrate diets physically limited voluntary intake, with restrictions in rumen fill (Harper & McNeill, 2015). On the other hand, higher intake of non-fibrous carbohydrates (NFCI) may have promoted energy satiety and limited intake in diets with 87% concentrate (Allen *et al*., 2009). As expected, higher concentrate levels and DMI improved body performance (Wang *et al*., 2024).

The NFCI increases the synthesis of propionate in the rumen, exceeding the liver's gluconeogenic capacity, promoting energy satiety (Allen *et al*., 2009), reducing the increment rate of DMI (from 96 to 3 g/day) among diets containing 59%, 73%, and 87% concentrate, time feeding, and meals in our study. In addition to energy satiety, animals favoring shorter meals – more and shorter meal durations – may also have evolved to reduce the flow of NFC per meal into the rumen, thereby controlling fermentation peaks and the fall in ruminal pH. Although DM and NFC intake per meal increased with increased concentrate intake, increasing the number of meals reduced the flow of these nutrients into the rumen by approximately 17%. However, our study did not evaluate ruminal fermentation parameters to elucidate these behavioral and nutritional dynamics.

In contrast, more concentrate increases the proportion of fine particles and the rate of passage through the gastrointestinal tract (Pinho *et al*., 2018), while reducing the contact time of the digesta with microorganisms and the digestibility of nutrients in the present study. High-grain diets have been reported to decrease ruminal pH and cellulolytic microorganisms (Kim *et al*., 2018; Zhang *et al*., 2017), resulting in the intense reduction in neutral detergent fiber digestibility (NDFD) in the present study.

In addition, the greater intake of fine particles and lower NDFI reduced the need for rumination and breaking down the physical barriers of the feed, which linearly decreased the chewing rate and the frequency of daily rumen boluses. Lower energy costs for animals during chewing and fiber digestion to meet nutritional energy requirements, leading to increased idleness time and greater energy availability to improve animal performance, corroborate the increase in ADG.

The increased DMI and reduced FET increased the animals' feeding and rumination efficiencies as the concentrate level in the diet increased, resulting in more DM ingested per unit time (kg DM/h). ndfFE and ndfRE showed better results with diets containing approximately 59% concentrate, which is directly related to the high fiber intake in this diet and confirms physical limitations in the ingestion and digestion of food in diets containing only 45% concentrate. ndfRE is related to NDFD and animal performance, as higher levels of NDFD require greater efficiency from microorganisms in the physical degradation of fiber and transformation into energy to be deposited as body weight gain (Argenta *et al*., 2019).

Physiological Variables

The increase in RR, HR, and RT occurs in response to the elevated metabolic rate promoted by the higher levels of concentrate, DMI, and NFCI. With the increased availability of gluconeogenic substrates, organs become metabolically more active, demanding more nutrients, thereby enhancing endogenous heat production (De *et al*., 2021; Pereira *et al*., 2022). De *et al*. (2021) reported a reduction in thermoregulatory rates in sheep subjected to food restriction and a decrease in DMI.

Although the effects of energy satiety on food intake are consistent, it is suggested that the limitation in DMI in diets with 87% concentrate may be related to the reduced flow of nutrients for metabolism and the synthesis of endogenous heat, which increases the idle time for the activation of thermoregulatory mechanisms for heat dissipation. Heat stress and increased thermoregulation rates reduce blood flow to the rumen, rumination, and appetite of animals, possibly leading to a reduction in DMI (Santos *et al*., 2019; De *et al*., 2024).

According to Talmón *et al*. (2023), fiber metabolism also contributes to internal heat production due to the intense muscular activity during rumination. However, this behavior was not observed in our study, as diets with more fiber resulted in lower thermoregulatory rates, particularly in those promoting greater NDIF, with 45% and 59% concentrate. In addition, the strong positive correlations between concentrate level and both RR and HR, along with the negative correlation between total chewing time and RT and HR, suggest that grain metabolism had a greater impact on thermoregulatory physiology than the muscular activity associated with chewing.

On the other hand, the lambs fed with 59% and 73% concentrate showed a reduction in the number of daily chews per ruminal bolus, while increasing the chewing time of each ruminal bolus, with a moderate positive correlation between NCb, RT, and RR. This behavior suggests that the animals' chewing activity may influence physiological parameters and that sheep might reduce the NCb as a strategic measure to lower endogenous heat, as indicated by the reduction in RT and RR. Corazzin *et al*. (2021) reported a reduction in the number of ruminal boluses during holidays subjected to heat stress, but it did not affect the rumination intensity of each ruminal bolus.

All animals in our study were under high stress, with RR ranging from 80 to 120 mpm (Silanikove, 2000). The evaluation times in our study showed high air temperature and THI, which justifies the high thermoregulatory rates found. During the afternoon, when THI exceeded 80, the RR of all lambs increased to over 100 mpm, classifying them as stage III of animal welfare. This stage involves reduced thermoregulatory pathway efficiency and potential failure (Silanikove, 2000), making it the most critical period for the animals' homeothermy. The decrease in feed intake and rumination during the afternoon, along with the increase in rumination and digestion during cooler hours (Figures 1 and 2), may indicate the ability of ruminant animals to adapt to hot climates (Nobre *et al*., 2016), shifting rumination, digestion, and endogenous heat synthesis activities to the cooler hours of the day (De *et al*., 2024).

Lambs fed 87% concentrate already presented stage III at 9:00 a.m. (FR = 107 mpm), confirming the strong effect of grain metabolism on disrupting homeostasis and welfare in animals kept in high-temperature environments. On the other hand, although the increase in concentrate raised physiological parameters, a diet with 73% concentrate promoted increased nutrient intake and body performance, along with a minimal increase in RT, due to the efficiency of the FR and HR pathways.

Our study has no practical limitations; however, evaluating ruminal, blood, and physiological parameters in respirometric chambers could enhance the study, providing a greater understanding of the interaction between nutritional parameters and thermoregulation in sheep. In addition to these parameters, future studies should explore new nutritional aspects, such as particle size and sources of non-fibrous carbohydrates.

CONCLUSION

High-concentrate diets increase nutrient intake and body performance in sheep, as well as the thermoregulatory rates of animals. Masticatory activity minimized changes in physiological parameters, whereas the proportion of grains in the diet was the main factor disrupting the animals' body homeostasis. Diets with 87% concentrate increase the heat stress level at all assessment times. Diets with 73% concentrate can be used for confined sheep in tropical regions with high temperatures, as they promote a slight increase in RT, while also increasing nutrient intake and body performance.

CONFLICT OF INTEREST

We certify that there is no conflict of interest with any financial, personal, or other relationships with other people or organizations related to the material discussed in the manuscript.

DECLARATION OF GENERATIVE AI AND AI-ASSISTED TECHNOLOGIES IN THE WRITING PROCESS

During the preparation of this work, the author(s) did not use AI-assisted technologies in the writing process.

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