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Canopy structure, behavioral and physiological aspects of pasture-finished sheep using castor bean cake as alternative input

[*Estrutura do dossel, aspectos comportamentais e fisiológicos de ovinos terminados em pasto, usando-se torta de mamona como insumo alternativo*]

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ABSTRACT

The purpose was to evaluate structural characteristics of pasture, behavioral aspects and physiological parameters of sheep finished on irrigated Tamani grass pasture under continuous stocking, using castor bean cake as alternative input. Structural characteristics of the pasture were assessed in a randomized block design in 2 x 2 factorial arrangement, with two sources of nitrogen fertilization (mineral - urea and organic - *in natura* castor bean cake) and two supplements (with or without detoxified castor bean cake), in four replications (paddocks). Behavioral aspects and physiological parameters were assessed in a randomized block design with four treatments, and repeated measures over time in eight replications (sheep). Total forage biomass, green leaf blade biomass, grazing, rumination, and idleness times, as well as respiratory rate and rectal temperature were assessed. Urea provided greater leaf blade biomass (1,807 kg DM.ha.cycle^{-1}) and tiller population density (1,592 tillers.m^{-2}). Grazing time reduced 65 minutes.day^{-1} for animals supplemented with detoxified castor bean cake and kept in pastures fertilized with urea. The use of castor bean cake did not change the physiological parameters of the animals in any of the managements evaluated and can be used as a strategic input in the finishing of sheep on pasture.

Key words: leaf area index; rectal temperature; *Ricinus communis*; Santa Inês

RESUMO

Objetivou-se avaliar as características estruturais da pastagem, os aspectos comportamentais e os parâmetros fisiológicos de ovinos terminados em pastagem irrigada de capim Tamani sob lotação contínua, utilizando-se torta de mamona como insumo alternativo. As características estruturais da pastagem foram avaliadas em delineamento em blocos ao acaso, em arranjo fatorial 2 x 2, com duas fontes de adubação nitrogenada (mineral - ureia e orgânica - torta de mamona in natura*) e dois suplementos (com ou sem torta de mamona desintoxicada), em quatro repetições (piquetes). Os aspectos comportamentais e os parâmetros fisiológicos foram avaliados em delineamento em blocos ao acaso, com quatro tratamentos e medidas repetidas no tempo em oito repetições (ovinos). Foram avaliados a biomassa total de forragem, a biomassa de lâmina foliar verde, os tempos de pastejo, a ruminação e o ócio, bem como a frequência respiratória e a temperatura retal. A ureia proporcionou maior biomassa de lâmina foliar (1.807kg MS.ha.ciclo^{-1}) e densidade populacional de perfilhos (1.592 perfilhos.m^{-2}). O tempo de pastejo reduziu 65 minutos.dia^{-1} para animais suplementados com torta de mamona desintoxicada e mantidos em pastagens fertilizadas com ureia. A torta de mamona não alterou os parâmetros fisiológicos dos animais em nenhum dos manejos avaliados, podendo ser utilizada como insumo estratégico na terminação de ovinos a pasto.*

Palavras-chave: índice de área foliar, Ricinus communis, *Santa Inês, temperatura retal*

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INTRODUCTION

Pastures are the cheapest source of feed in ruminant farming. In Brazil, this activity is predominantly extensive, which gives it one of the lowest costs of meat and milk production in the world. To maximize the productive potential of pastures, new forage species are being developed and constantly evaluated in intensive systems, especially with nitrogen fertilization (Vasconcelos *et al*., 2020; Schons *et al*., 2021).

The Tamani cultivar (*Megathyrsus maximus* cv. BRS Tamani) was the first hybrid released by the Brazilian Agriculture Research Corporation - Embrapa, and stands out for its short height, emergence of leaves and tillers, high nutritional value, and adaptation to the edaphoclimatic conditions of tropical regions. Although it is an interesting alternative from the point of view of plant production, the set of management strategies adopted and their relationships (soil-plant-animal) can interfere in the morphological components, which will influence the structure of the pasture and, consequently, the forage production, use efficiency, and animal performance (Silva *et al*., 2018; Schons *et al*., 2021).

Understanding these relationships, combined with the use of supplementation, favors greater gains per area and improves animal performance by promoting adequate amounts of nutrients not available in forage, optimizing the digestibility of the pasture and the intake by the grazing animals (Hodgson, 1990). However, according to Mendes *et al*. (2015) supplementation can reduce the DM intake by the animal, altering their ingestive behavior while grazing, due to the substitution effect, and can also increase the cost of production systems.

The use of alternative inputs can help mitigate animal supplementation costs. With that in mind, the castor bean cake is a by-product obtained from oil extraction and has interesting nutritional value and digestibility, however it contains toxic substances such as ricin and ricinus agglutinin according to Dang and Vam Damme (2015), which prevents its use in the *in natura* form in animal feeding. However, safe and economically feasible detoxification methods already exist (Andrade *et al*., 2019), with promising reports of its use in animal production (Santos Neto *et al*., 2019; Araújo *et al*., 2020).

Castor bean cake has been used as a natural fertilizer because it is a nutrient-rich product that presents interesting contents of nitrogen, phosphorus, potassium, calcium and micronutrients, in addition to providing a positive effect on the control of soil nematodes and the free-living stage of gastrointestinal parasites in grazing sheep (Severino *et al*., 2012; Maranguape *et al*., 2020).

In this context, the purpose of the present study was to evaluate the effects of detoxified castor bean cake replacing the soybean meal in the feed and *in natura* castor bean cake replacing urea as fertilizer, on the structural characteristics of Tamani grass canopy, the behavioral aspects, and physiological parameters of sheep on irrigated pasture under continuous stocking.

MATERIAL AND METHODS

All procedures were approved by the Ethics Committee on the Use of Animals (CEUA) of Embrapa Goats and Sheep, under protocol No. 001/2017.

The study was carried out at Três Lagoas Farm, which belongs to Embrapa Goats and Sheep in the city of Sobral - CE, Brazil, located at latitude 3º44’50” South and longitude 40º21’28” West, from October 2019 to January 2020. The climate of the region is classified as BSh, warm semi-arid (Köppen, 1936). The climatic data were collected by the weather station installed in the experimental area, during the experimental period. The averages of 29.57ºC, 66.54%, 1,881.03µmol m^{-2} s^{-1}, 7.75 mm.day^{-1}, were recorded for temperature, humidity, radiation, and precipitation, respectively.

The treatments tested involved the use of castor bean cake as an alternative input in the sheep diet replacing soybean meal and as an organic nitrogen fertilizer replacing urea, which were: sheep supplemented with feed containing soybean meal and pasture fertilized with urea (SMUR); sheep supplemented with feed containing detoxified castor bean cake and pasture fertilized with urea (CCdUR); sheep supplemented with feed containing soybean meal and pasture fertilized with *in natura* castor bean cake (SMCC); and sheep supplemented with feed containing detoxified castor bean cake and pasture fertilized with in natura castor bean cake (CCdCC). For the behavior and physiological evaluations, a randomized block design with four treatments (SMUR, CCdUR, SMCC and CCdCC) was used, under repeated measures over time in eight replications (sheep), while for the evaluation of the structural characteristics of the Tamani grass pasture, it was used a randomized block design with a 2 x 2 factorial arrangement, evaluating two sources of nitrogen fertilization (mineral in the form of urea and organic in the form of *in natura* castor bean cake) and two supplements (with and without detoxified castor bean cake), by distributing four animals per paddock in four replications (paddocks), totaling 16 sheep per treatment

Sixty-four Santa Inês sheep were used, 32 castrated males and 32 females, at an average age of 3.60 ± 0.60 months and initial weight of 19.42 ± 3.60kg. Eighteen ewes were used as balance animals, with body weight (BW) of 35.00 ± 3.53 and approximately five years of age, also managed under continuous stocking, and receiving concentrate supplementation, according to the test animals.

Soil was sampled in a layer with 0-20cm of depth for the evaluations of physical and chemical attributes. The soil of the experimental area was classified as Ortic Chromic Luvisol according to Santos *et al*. (2018) and presented the following characteristics: pH= 6.8; Organic Matter= 17.3g.kg^{-1}; P=23mg. kg^{-1}; K=0.2cmolc.kg^{-1}; Ca=11.5cmolc.kg^{-1}; Mg=3.4cmolc.kg^{-1}; H+Al= 2.0cmolc.kg^{-1}; Al=0.0 cmolc.kg^{-1}; Sum of bases (SB)=15.1 cmolc.kg^{-1}; Cation exchange capacity (CEC)=17.1 cmolc.kg^{-1}; and V=89%. The soil presented for S, Na, Cu, Fe, Zn, Mn, and B the values of 153, 23, 40, 80, 13, 159 and 1.3cmolc.kg^{-1}, respectively. And the values obtained for clay, silt; coarse sand and fine sand were 161, 219, 327 and 293g.kg^{-1} respectively.

Based on soil analysis, foundation fertilization was performed with the formulation 06:28:16 to meet the recommendation of 40:70:40kg of NPK, plus 40kg.ha^{-1} of micronutrient FTE BR-12 (Cantarutti *et al*., 1999). Previously, the area went through mechanized crop treatments: cutting, plowing, and harrowing. The tamani grass pasture was implemented on July 12, 2019, with the aid of a four-row hydraulic planter. An amount of seeds equivalent to 20kg.ha^{-1} was used, which was sown at 40cm between rows and 2cm deep.

The area was provided with low-pressure fixed sprinkler irrigation system, with service pressure < 2.5kgf cm^2. Irrigation was performed daily, during the night period. The applied water corresponded to an average crop evapotranspiration of 6.9mm.day^{-1}, with application efficiency of 75%. The evaluation of uniformity of water distribution by the system was performed with rain gauges spaced at 3.0 x 3.0m, height of 0.5m from the ground, in two diagonally alternated paddocks.

The *in natura* castor bean cake used as organic fertilizer showed values for N, P, K, Ca, Mg, and S of 55, 12, 15.7, 6.5, 8.7, and 1.6g.kg^{-1} and C:N ratio of 5.2, while for Cu, Fe, Zn, Mn and B the values were 26, 532, 168, 62 and 7mg.kg^{-1}. The castor bean cake destined for animal supplementation was detoxified with calcium oxide and it was performed the electrophoretic characterization (SDS-PAGE) of the samples of *in natura* castor bean cake extract (*in natura* CC), detoxified castor cake (CCd) and the diet containing CCd (DCCd), in addition to the analysis of the hemagglutinating activity of CC lectins according to Andrade *et al*. (2019).

The Tamani grass pasture was fertilized according to the treatments with urea (45% N) or *in natura* castor bean cake (5% N). The recommendation of 45kg.ha^{-1}.year^{-1} was followed, according to Vasconcelos *et al*. (2020) and the pasture was managed at an average height of 22cm. A total of 66kg of urea and 592kg of *in natura* castor bean cake per cycle.ha^{-1}.year^{-1} was used for the chemical and organic fertilizations. Both applications were equally fractioned, being applied at the beginning (12 days) and in the middle (12 days) of the productive cycles of the crop, which were 24 days each. The pasture was managed under continuous stocking with variable stocking rate (Mott and Lucas, 1952). The total area corresponded to 1 ha, divided into 16 plots of 500m² each, for grazing by the experimental animals and two plots for allocation of the balance animals. All paddocks were delimited by a wire mesh fence, provided with feeders, drinking troughs, salt troughs and 2.0 x 3.0m shading screens, with 50% light transmittance.

The male sheep were castrated with a "*burdizzo*" castrator at the beginning of the experiment, in accordance with CFMV Resolution No. 877 of February 2008 (Conselho…, 2008). Before being allocated to the paddocks, the animals were treated with antiparasitic agents based on 10% Closantel sodium (10mg.kg^{-1}) and 5% levamisole hydrochloride (5mg.kg^{-1}), and the absence of infection by gastrointestinal parasites was confirmed through EPG count.

The diets based on corn, soybean and detoxified castor bean cake were formulated for an average daily body weight gain of 200g.day^{-1}, in the proportion of 14% crude protein (CP) and 67.9% of total digestible nutrients (TDN), according to Miranda (2018), to offer a diet of greater resilience to parasites to the grazing sheep, reducing the effects caused by hemoncosis. The chemical composition of the ingredients and their proportions are shown in Tab. 1, 2 and 3.

Table 1. Chemical composition of ingredients of the experimental diets

Items (g.kg^{-1} Dry matter)	Ingredients				
	TGu	TGc	GC	SM	CCd
Dry Matter	954.80	956.80	889.90	902.40	931.20
Organic matter	877.50	874.30	932.10	985.10	846.40
Mineral matter	122.50	125.70	18.50	14.90	153.60
Crude protein	101.30	101.40	101.50	489.40	301.30
Neutral detergent insoluble protein	38.10	40.30	25.00	30.50	129.40
Acid detergent insoluble protein	10.00	7.30	20.60	33.10	81.30
Ether extract	36.40	31.60	58.00	43.80	78.30
Total carbohydrates	739.80	741.30	822.00	451.90	466.80
Non-fiber carbohydrates	16.80	3.80	659.60	271.4	136.60
Structural carbohydrates	720.30	737.50	163.00	180.50	330.20
Neutral detergent fiber	758.10	764.70	191.90	192.90	355.20
NDF corrected for ash and protein	723.00	737.50	163.00	180.50	330.20
Acid detergent fiber	366.70	372.00	54.00	39.70	328.50
Lignin	13.30	15.70	6.10	1.30	36.00
Hemicellulose	391.40	392.70	137.90	153.20	26.70
Total digestible nutrients	574.70	565.20	861.30	853.60	695.20

TGu – Tamani grass fertilized with urea; TGc - Tamani grass fertilized with *in natura* castor bean cake; GC – Ground corn; SM – Soybean meal; CCd – detoxified castor bean cake.

Table 2. Proportions of the ingredients of the experimental diets effectively consumed

	Proportions of ingredients	
	Standard Feed	Alternative Feed
Item (% dry matter)	al diet	
Tamani grass	51.13	6.95
Ground corn	39.10	3.42
Soybean meal	9.77	---
Detoxified castor bean cake	----	6.45
Soybean oil	----	.18
Mineral salt[1]	t will	
Total	100	00

Standard feed: based on ground corn and soybean meal; Alternative feed: based on ground corn and castor bean cake.
[1]Composition: phosphorus - 65.00 g; calcium - 177.50 g; sulfur - 20.00 g; magnesium - 8.00 g; sodium - 162.00 g; cobalt - 0.04 g; zinc - 1.90 g; manganese - 1.35 g; iodine - 0.071 g; selenium - 0.02 g; fluorine - 0.76 g; copper - 0.20 g and vehicle - 1,000 g.

Table 3. Chemical composition of the experimental diets

Items ($g.kg^{-1}$ Dry matter)	Chemical composition of the diet	
	Standard Feed	Alternative Feed
Dry Matter	924.80	931.00
Organic matter	908.50	862.00
Mineral matter	72.10	89.70
Crude protein	139.30	131.10
Neutral detergent insoluble protein	32.80	48.00
Acid detergent insoluble protein	16.40	20.40
Ether extract	44.30	79.70
Total carbohydrates	744.20	699.20
Non-structural carbohydrates	289.50	247.50
Structural carbohydrates	454.70	451.60
Neutral detergent fiber	483.20	480.00
NDF corrected for ash and protein	454.70	451.60
Acid detergent fiber	213.80	245.50
Lignin	9.90	14.80
Hemicelluloses	269.30	234.50
Total digestible nutrients (Nutrient…, 2001)	711.60	735.60

Standard feed: based on ground corn and soybean meal; Alternative feed: based on ground corn and castor bean cake.

The supplement was offered in the proportion of 1.8% of BW, considering a daily dry matter intake equivalent to 3.6% of BW (Nutrient…, 2007). The mineral salt was supplied at will in the morning, and the concentrated supplement was supplied daily at 17:30 pm, which was the best time due to the lower activity of Africanized bees (*Apis mellifera*), present in the troughs.

The average height of the pasture (HEI) was recorded daily, with the aid of a retractable graduated stick, by sampling thirty-five spots per paddock. The assessment of leaf area index (LAI) and photosynthetically active radiation interception (PAR/LAI) were performed using a PAR/LAI analyzer in Agriculture model Accupar LP-80 (Decagon Devices®). Twelve readings were taken at representative spots of each experimental unit (paddocks), by taking six readings (internal and external) from each exclusion cage, with an area of 0.5 x 0.5m^2. The relative chlorophyll index (RCI) was measured in eight newly expanded leaves per paddock by a chlorophyll meter (Chlorophyll Meter SPAD-502)

The tiller population density (TPD) was estimated by counting all live tillers within two 0.5 x 0.5m frames in each paddock. At the end of each growth cycle, the number of live leaves per tiller (NLL) was determined by counting the number of newly expanded leaves of five intact tillers within two exclusion cages, allocated to each paddock. For this count, a value of 1.0 was assigned for expanded leaves and 0.5 for emerging leaves.

At the end of each cycle, the biomasses of total forage (TFB), green forage (GFB), green leaf blade (GLB), green stem (GSB), as well as the live/dead material (LM/DM) and leaf blade/stem (LB/S) ratios, which were estimated from the average of two samples collected with the same frame used to count the tiller population density (TPD), by cutting the forage close to the ground in each paddock. Later, in the laboratory, the samples were fractioned and placed in perforated paper bags, weighed, identified, and dried in a forced ventilation oven at 55°C until reaching constant weight, and then weighed again.

The behavior trial was carried out on December 19, 2019, over 24 hours, always with the same, previously trained observers. Two observers were assigned to each of the four paddocks, taking turns of three-hour shifts. The day before the trial, eight lambs from each treatment were enumerated on the back and sides with spray paint to aid in identification during the evaluation. For a week during the night, observers went to the paddocks to accustom the animals to their presence and to the presence of the light from flashlights used to assist in viewing the animals during the trial, when necessary.

The behavior assessment started at 6am, and the evaluations consisted of two measurement ways, with the continuous activities (grazing time, rumination time, idleness time or other activities) recorded every 10 minutes and the punctual activities (defecation, urination, water intake, and salt intake) recorded each time the animals performed it. The data were tabulated at six-hour intervals. Thus, four evaluation periods were obtained (6am-12pm; 12pm-6pm; 6pm-12am; 12am-6am). The data referring to continuous activities were tabulated in minutes of the total time of each six-hour interval. On the other hand, punctual activities were tabulated as frequency (number of times that each animal, on the average of the eight, performed such activity during the six-hour interval).

The physiological parameters: respiratory rate (RR), heart rate (HR), rectal temperature (RT) and body surface temperature (BST) were measured, in this order, to reduce the influence of the stress of physical restraint, during three consecutive days, at the following times: from 6am to 8am and from 12pm to 2pm. The RT, expressed in Celsius degrees, was determined by a digital clinical thermometer (Techline® TS-101PM) introduced directly into the rectum until stabilization. A non-contact digital infrared thermometer (ICEL-Manaus®- TD-950) was used to obtain the BST, which was the arithmetic mean of the temperatures obtained in five regions of the body: surface temperature of the forehead (FST), neck (NST), loin (LST), back (BST), and belly (BST). The HR was obtained with the aid of a stethoscope positioned on the left side of the animal in the cardiac region (3rd-4th intercostal space), while the RR was measured by counting the number of flank movements. In both measurements, the evaluation time was 15 seconds, and the obtained value was multiplied by four to express them per minute (beats/minute and movements/minute). Fig. 1 shows the weather data collected from a weather station installed in the experimental area during the period of behavioral and physiological evaluations.

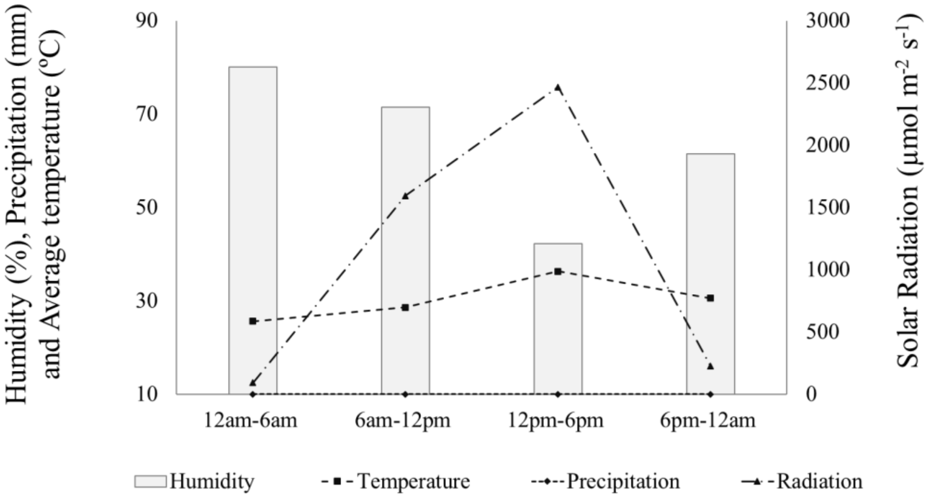


Figure 1. Air relative humidity (%), rainfall precipitation (mm), average temperature (ºC), and solar radiation (µmol m^{-2} s^{-1}) during the period of behavioral and physiological evaluations.

The data were subjected to normality (Shapiro-Wilk) and homoscedasticity (Levene) tests and, once the assumptions were met, they were subjected to analysis of variance through the F test. The interaction diet x fertilization was unfolded only when significant at 5% probability. To evaluate the effects of diet and fertilization, the means were compared through the Tukey's test at 5% probability. Statistical analyses were performed using the GLM procedure of the SAS software version 9.4 (SAS, 2005), according to the model:

For behavioral aspects and physiological parameters:

$$Y_{ijkl} = \mu + \propto_j + \beta_k + (\propto\beta)_{jk} + \gamma_{jk} + \delta_l + (\propto\delta)_{jl} + (\beta\delta)_{kl} + (\propto\beta\delta)_{jkl} + \varepsilon_{ijkl},$$

Where, Y_{ijkl} = value of the i^{th} experimental unit, that received the treatment combination referring to the j^{th} diet and k^{th} fertilization evaluated in the l^{th} repeated measure; μ = effect of general mean; α_i = effect of diet; β_j= effect of fertilization; $(\alpha\beta)_{ij}$ = effect of interaction between diet and fertilization; γ_{jk} = random effect of the error associated to the experimental units; δ_l = effect of repeated measure; $(\propto\delta)_{jl}$ = effect of interaction between diet and repeated measure; $(\beta\delta)_{kl}$ = effect of interaction between fertilization and repeated measure; $(\propto\beta\delta)_{jkl}$ = effect of interaction between diet, fertilization and repeated measure; ε_{ijkl} = effect of random error associated to each observation, assuming a normal distribution.

For the structural characteristics of the pasture:

$$Y_{ijk} = \mu + \gamma_i + \propto_j + \beta_k + (\propto\beta)_{jk} + \varepsilon_{ijk},$$

Where, Y_{ijk} = value of the experimental unit in the i^{th} block, that received the treatment combination referring to the j^{th} diet and k^{th} fertilization; μ = effect of the general mean; γ_i = effect of block; α_i= effect of diet; β_j= effect of fertilization; $(\alpha\beta)_{ij}$= effect of interaction between diet and fertilization; ε_{ijk} = effect of random error, assuming a normal distribution.

RESULTS

Considering the fertilizer factor, effects were observed on the variables LAI (P<0.01), LI (P<0.01), HEI (P<0.01), TPD (P<0.01), NLL/tiller (P<0.01), LM/DM (P<0.01), GFB (P<0.05), GLB (P<0.05) and LB/S ratio (P<0.05). No effect (P>0.05) was observed on CRI and TFB (Tab. 4). The LAI values ranged from 2.12 to 2.52, being higher when the animals were supplemented with CCd and grazed in pastures fertilized with urea. In addition, fertilization with urea provided increases of 15.90, 7.61 and 4.67% in LI, HEI and NLL/tiller, when compared to organic fertilization (*in natura* CC). The highest tiller population density, which was 1,592 $tillers.m^{-2}$, was found in pastures fertilized with urea. The CRI showed a mean value of 23.36 unit.SPAD. The mean TFB value among treatments was 3,723.40kg $DM.ha.cycle^{-1}$ with no effect of treatment (P>0.05). The pasture fertilized with UR showed the highest yield of GFB and GLB, which were 2,758 and 1,807kg $DM.ha.cycle^{-1}$.

Table 4. Structural characteristics of irrigated tamani grass managed under continuous stocking, using castor bean cake as supplement replacing soybean meal or as organic fertilizer replacing urea

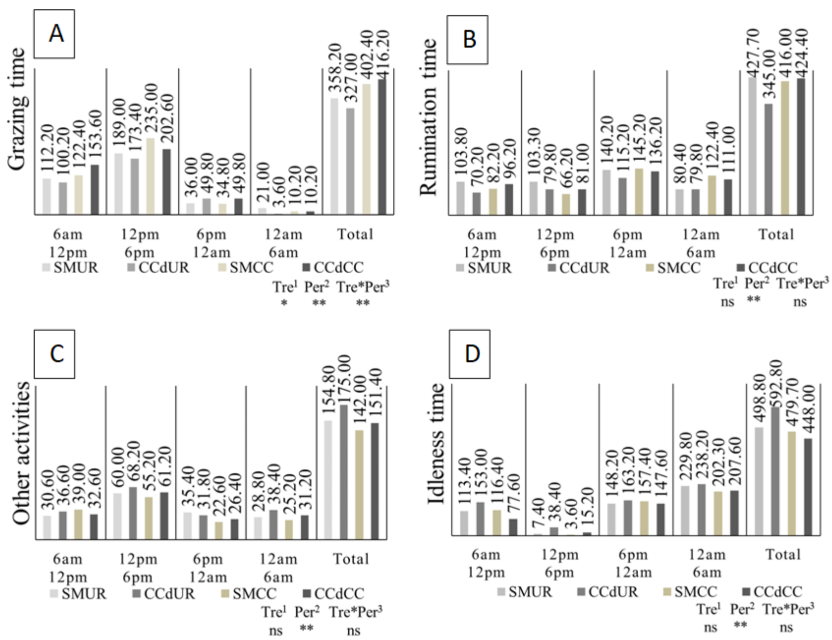
	Treatments							
Variables	Feeds		Fertilizers		EM[1]	P-value		
	SM	CCd	UR	CC		Supl[2]	Fert[3]	Supl*Fert[4]
LAI	2.17A	2.47B	2.52a	2.12b	0.06	**	**	ns
LI	62.77	67.61	68.89a	61.48b	1.56	ns	**	ns
CRI	23.26	23.26	23.79	23.14	0.27	ns	ns	ns
HEI	22.03B	22.96A	23.38a	21.60b	0.26	*	**	ns
TPD	1,532	1,484	1,592a	1,420b	6.32	ns	**	ns
NLL	2.15	2.04	2.14	2.04	0.05	ns	ns	ns
TFB	3,822	3,625	4,040	3,407	208.35	ns	ns	ns
GFB	2,450	2,425	2,758a	2,117b	159.73	ns	*	ns
GLB	1,473	1,621	1,807a	1,287b	119.95	ns	*	ns
GSB	976.30	804.0	950.20	830.10	59.73	ns	ns	ns
LM/DM ratio	1.90	1.97	2.08a	1.78 b	0.06	ns	**	ns
LB/S ratio	1.70B	2.05A	1.99a	1.77 b	0.06	**	*	ns

LAI - leaf area index; LI - light interception; CRI - chlorophyll relative index; HEI - pasture height; TPD - tiller population density; NLL/tiller - number of live leaves/tiller; TFB - total forage biomass; GFB - green forage biomass; GLB - green leaf blade biomass; GSB – green stem biomass; LM/DM - live material/dead material ratio and LB/S - leaf blade/stem ratio. SM - Soybean meal; CCd- detoxified castor bean cake; UR - Urea; CC - *in natura* castor bean cake. Means followed by different uppercase letters on the rows between feeds and lowercase letters on the rows between fertilizers were statistically different according to the Tukey's test (P<0.01**; P<0.05*; non-significant[ns]). [1]Standard error of the mean; [2]Effect of diet; [3]Effect of fertilizer; [4]Effect of the interaction between diet and fertilizer.

It was observed effect of the diet on the variables leaf area index (P<0.01), light interception (P<0.01), height (P<0.05) and leaf blade / stem ratio (P<0.01). The other variables had no effect (P>0.05) (Table 4). LAI and LB/S ratio showed values of 2.47 and 2.05, respectively, in pastures where the animals were supplemented with the diet containing detoxified castor bean cake (CCd), while in the pasture where the animals were supplemented with the diet based on soybean meal (SM), the values were 2.17 and 1.70, respectively.

For continuous behavioral variables, there was effect of interaction (treatments x period) only on grazing time, where the value of 327 minutes.day^{-1} was observed for sheep kept on pastures fertilized with urea and supplemented with the diet containing detoxified castor bean cake (CCdUR), which was lower than the others during the daytime (Fig. 2A). While animals supplemented with the diet containing CCd and kept on pasture fertilized with urea (CCdUR) had a reduction of 65 minutes.day^{-1} in grazing time when compared to the average of the other management systems (392.2 minutes.day^{-1}).

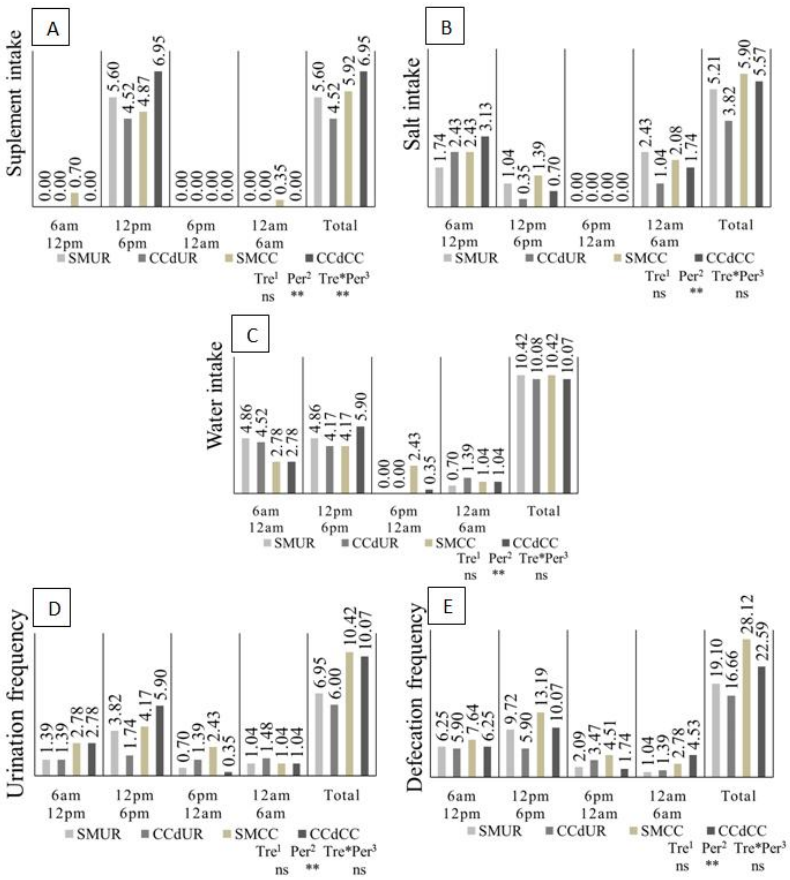
The variables rumination time, other activities, and idleness (Fig. 2B, C and D) had effect (P<0.01) only on the period factor. On average, the time spent by animals on rumination was 403 minutes.day^{-1}, which was concentrated in the night period. The average time spent by the animals on other activities and idleness was 155.8 and 504.8 minutes.day^{-1}, which was concentrated in the intervals 12pm-6pm and 12am-6am, respectively.



Means followed by different uppercase letters for treatments and lowercase letters for periods are statistically different according to the Tukey's test P<0.01 (**), P<0.05 (*) and non-significant (ns) (P>0.05). [1]Effect of treatment; [2]Effect of period; [3]Effect of interaction between treatment and period.

Figure 2. Activities performed (minutes.day^{-1}) by sheep kept on irrigated Tamani grass pasture under continuous stocking, using castor bean cake as supplement replacing soybean meal or as organic fertilizer replacing urea.

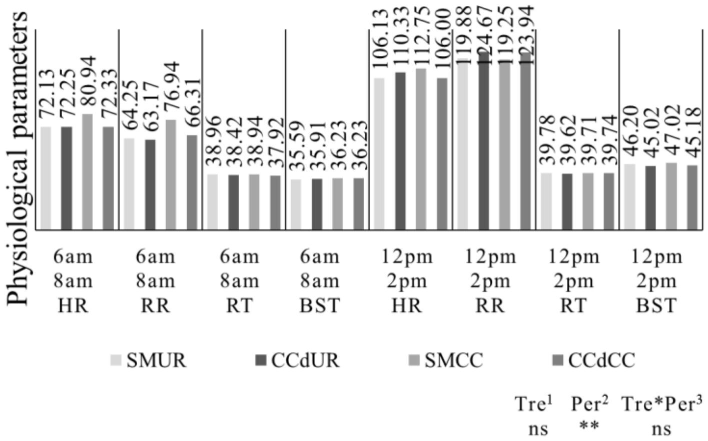
For the punctual variables, there was effect of interaction (treatment x period) only on the frequency of concentrated supplement intake (Fig. 3A). The highest frequency of concentrated supplement intake coincided with the interval of 12pm-6pm, due to the time of supply (5:30pm). Mineral salt intake was observed from 12am-6am (Fig. 3B). The frequency of water intake was more expressive in the intervals between 6am-12pm and 12pm-6pm (Fig. 3C). There was effect (P<0.01) of period on the punctual activities, urination, and defecation, which were more frequent in the intervals of greater grazing frequency, from 12pm-6pm (Fig. 3D and E).



Means followed by different uppercase letters for treatments and lowercase letters for periods are statistically different according to the Tukey's test P<0.01 (**), P<0.05 (*) and non-significant (ns) (P>0.05). [1]Effect of treatment; [2]Effect of period; [3]Effect of interaction between treatment and period.

Figure 3. Activities performed (number of times.animal.day^{-1}) by sheep kept on irrigated Tamani grass pasture under continuous stocking, using castor bean cake as supplement replacing soybean meal or as organic fertilizer replacing urea.

The physiological variables: heart rate (HR), respiratory rate (RR), rectal temperature (RT) and body surface temperature (BST) were affected only by the period, showing mean values of 109.00 beats.minute^{-1}, 122 movements.minute^{-1}; 39.70 and 45.85ºC, which were higher in the afternoon, from 12pm-2pm. While the lowest values, which were 74 beats.minute^{-1}, 68 movements.minute^{-1}, 38.55 and 35.98ºC, were seen in the morning from 6am-8am (Fig. 4).



HR - heart rate (beats/minute); RR - respiratory rate (movement/minute); RT - rectal temperature (ºC) and BST - body surface temperature (ºC). Means followed by different uppercase letters for treatments and lowercase letters for periods are statistically different according to the Tukey's test P<0.01 (**), P<0.05 (*) and non-significant (ns) (P>0.05). [1]Effect of treatment; [2]Effect of period; [3]Effect of interaction between treatment and period.

Figure 4**.** Evaluation of physiological parameters of sheep kept on irrigated Tamani grass pasture under continuous stocking, using castor bean cake as supplement replacing soybean meal or as organic fertilizer replacing urea.

DISCUSSION

The increase in LAI in the pasture fertilized with UR is related to the higher TPD and GLB (Table 4), which are influenced by the chemical nitrogen fertilization (Martuscello *et al*., 2015). Urea is a chemical fertilizer that has a high nitrogen content and high solubility, being readily available to the plant, which contributed to a rapid increase in the NLL/tiller and LAI (Pearson and Ison, 1997), optimizing the LI and, consequently, the photosynthetic capacity of the canopy, with increases in GFB, GLB, LM/DM and LB/S ratio.

Similar response for the LAI variable was observed in pastures where the sheep were supplemented with a diet containing CCd. It is assumed that the reduction in the average grazing time, particularly for the CCdUR treatment, may have contributed to the result. This reduction in grazing time may be related to the lower dry matter digestibility (DMD - 62.71%) of the CCd when compared to the SM supplement (68.58%), due to the higher acid detergent fiber (ADF) and ether extract (EE) in the diet. In part, the higher ADF and lignin contents in the diet containing CCd, associated with the higher EE content influenced the lower DMD. The average presence of 15% of husk in the castor bean cake during the oil extraction process increased the levels of NDF and ADF of this feed. The nutritional value of the byproducts of oil extraction from castor seeds depends directly on the extraction method, species, degree of seed decortication (removal of seed husk) and processing of the resulting product.

According to Jung and Deetz (1993), lignin is contained in the ADF fraction, corresponding to the non-digestible part of plants, and is considered the main limiting factor of digestibility. In addition, the higher ether extract content in the diet containing CCd may also have contributed to reduce DM digestibility, mainly due to the inhibitory effect caused to rumen cellulolytic microorganisms, affecting their activity (Jenkins and Harvatine, 2014).

The chlorophyll relative index (CRI) generates information associated with the quantification of nitrogen in the leaves (Mengel and Kirkby 2001), which are associated with the photosynthetic potential of the plants. Both organic and mineral fertilization ensured an equitable contribution of nitrogen to the grass, obtaining similar yields of TFB (kg DM.ha.cycle^{-1}) among the treatments evaluated. The C:N ratio (5.2) of *in natura* CC used in the experiment contributed positively to the response, because low C:N ratio increases mineralization and nutrient availability by microorganisms to the plant.

Tamani grass showed phenotypic plasticity favorable to the recommended management of pasture under continuous stocking with sheep, presenting stability in the tiller population during the entire experimental period for both inputs evaluated. The higher tiller population density observed in pastures fertilized with urea reflects the intense activation of meristematic tissues, which interacting with water availability and light intensity promoted increase in the flow of plant organs (Duru and Ducrocq, 2000), reflecting in increased leaf elongation rate (Pompeu *et al*., 2010; Alexandrino *et al*., 2004), with consequent increase in TPD and leaf area.

Nevertheless, the TPD quantified in this study in the same condition of fertilization performed by Vasconcellos *et al*. (2020), who evaluated the morphogenetic and structural characteristics of tamani grass under increasing levels of nitrogen, was 35.54% lower. This lower TPD average is due to the planting spacing adopted by those authors, which was 22cm, in relation to the 40 cm used between rows in our study, which was the minimum spacing of the planter, in addition to the influence of grazing and trampling by animals on the grass. The LM/DM ratio was higher in pastures fertilized with UR, which is explained by the increase in GFB, especially due to the increase in GLB. Regardless of the nitrogen input used, nitrogen acts in the increase of cell production (cell division), which is the reason for this nutrient to directly influence the leaf elongation rate, by increasing the number of cells. Little nitrogen is deposited outside the cell elongation zone, indicating that RUBISCO synthesis depends on this nitrogen accumulation in the cell division zone, therefore, nitrogen deficits can compromise future photosynthetic efficiency (Skinner and Nelson, 1995), bringing consequences for growth and forage yield.

The LB/S ratio is an important indicator of forage quality and the higher the ratio, the higher the protein and digestibility levels, therefore, directly influencing the ingestive behavior and performance of grazing animals. The higher LB/S values observed in pastures fertilized with urea where the animals received the diet containing CCd (CCdUR) may be related to the higher GLB and the indirect effect of the shorter grazing time and consequent less manipulation of forage and trampling in these pastures. The reduction in grazing time was 65 minutes.day^{-1}, when compared to the average of the other management methods adopted (Fig. 2A). Thus, it can be said that the taller canopy height and the higher production of GLB contributed positively to the bite size, maximizing forage intake by the animal, and reducing grazing time. According to Hodgson (1990), the bite mass is directly related to the pasture structure, in particular canopy height, and the larger it is, the shorter the grazing time (Santos *et al*., 2010).

Furthermore, the higher level of ether extract (7.97%) in the CCd-based diet (Table 3), associated with the presence of ricinoleic acid contained in the by-product may have influenced the response, because there was reduction in DMD. In addition, EE levels above 7% DM negatively interfere with the activity of cellulolytic microbiota and rumen fermentation, reducing feed digestibility and passage rate (Nutrient…, 2001; Azevêdo *et al*., 2011; Alves *et al*., 2017; Barbosa *et al*., 2021). The reduction in grazing time of sheep supplemented with feed containing CCd in pastures fertilized with urea may have characterized a substitution effect, which is often observed in well-managed pastures with animals receiving energy supplementation, but without decrease and effect (P>0.05) on dry matter intake, nutrient intake, and pasture stocking rate in the present study. According to Poppi and Mclennan (1995), energy supplementation has a greater potential response in high-quality pastures where there is an excess of soluble nitrogen compounds due to intensive pasture management (nitrogen fertilization). In this case, the use of energy supplementation provides additional energy for better synchronization of microbial protein synthesis, reducing losses and rumen ammonia concentration.

The grazing peak was concentrated from 12pm-6pm, where it was observed *in loco* that, routinely, the sheep started grazing at 8:30am. It is inferred that at dawn the pasture was still wet, due to the daily night irrigation, leading to reduced grazing activity in part of the morning period, predominantly in the hours of the day of milder temperatures (Gregorini, 2012). In addition, the animals were receiving protein-energy concentrate in intensively managed pastures and, despite the high contents of NDF of the tamani grass (76.14%), it has a low content of ADF (36.93%), which is a fiber of high digestibility, that ensures the daily nutritional requirements of the sheep, also contributing to the satiation of these animals. According to Baumont *et al*. (2000), rumen distension caused by the ingested forage, associated with acetate production, are the factors that most contribute to satiety of the animals. However, the supply of concentrate with high proportion of non-fiber carbohydrates in the dry matter (59%), may have interfered with the digestion of the pasture fiber (Macedo Júnior *et al*., 2007), leading to reduced rumen pH and negative effect on fiber digestion, resulting from the preference of microorganisms for NFC, through the overlap of amylolytic bacteria over those that digest fiber, in competition for the same substrates or for enzymes that degrade fiber, and that can be inhibited by NFC or the products of their digestion (Hoover, 1986). Grazing was substituted by rumination, other activities and idleness, and the time spent by the animals in the interval 6am-12pm was relevant (Fig. 2B, C and D).

The time spent on rumination was distributed in 42.37% during the day and 57.63% at night, predominantly in the period between 6pm and 6am. Rumination is influenced by the type of diet and is directly related to the cell wall content of the roughage feed (Van Soest *et al*., 1991). In this study, it can be stated that the changes observed for the structural variables of the pastures did not compromise the NDF content of the sheep's diets, which was on average 48.16% (Table 3), and did not affect the rumination time (Fig. 2B).

The time spent on other activities (playing, walking, and observing) was more expressive from 12pm to 6pm, especially at dusk (Fig. 2C). The act of the animals running around the paddocks was possibly stimulated by the fact that they were already satiated, due to the greater concentration of time spent on grazing and to the offer of concentrated supplement, which occurred 5:30pm, associated with the better thermal sensation (Fig. 1). Thus, as the animals became satiated, much of their time was spent on other activities (Howery *et al*. 1998).

The time spent on idleness was greater from 12am-6am, representing 43.47% of the total time spent in the activity. However, an atypical behavior was observed for this activity from 6am-12pm (Fig. 2D). At that time, the animals remained idle 22.77% of the time, which can be explained by the daily night irrigation performed at 11:30pm, as previously reported. It was observed that the animals waited for the wet pasture to dry, to start their daily activities, mainly grazing, which was predominant in that period (Gregorini, 2012). Thus, it is suggested to stagger irrigation in shifts of up to three days, since the soil has medium texture ($161g.kg^{-1}$ clay), allowing greater water retention in the soil and increase the amount of irrigation, aiming to avoid the daily idleness of the animals in the morning, which is a period of great relevance to optimize the grazing time (Pompeu *et al*., 2009), allowing higher rates of gain. Moreover, such management can reduce the daily displacement of infective larvae in the pasture caused by the daily irrigation, preventing the increase of parasitic infection on sheep. In this context, it is of fundamental importance to evaluate the soil moisture retention curve, allowing the estimation of water storage capacity in the soil, according to the soil depth considered.

The change in the ingestive behavior of animals observed for the variables salt intake, and water intake between 12am and 6am, and concentrated supplement intake between 6am and 12pm was motivated by the presence of Africanized bees (*Apis mellifera*) in the area, with high visitation to the troughs, which inhibited the complete consumption of the supplement that was supplied at 5:30pm, particularly in the SMCC treatment (Fig. 3B and C). The highest frequency of water intake was seen from 6am-12pm and 12pm-6pm, coinciding with the high temperatures, low humidity, and grazing peak (Fig. 1 and 2). According to Santos *et al*. (2011), this variable correlates positively with temperature, being important in the recovery of water lost by tissue evaporation and by breathing. Associated with the climatic conditions, the increase in grazing and concentrate consumption observed at these intervals contributed to an increase in the frequency of water intake. Also, the DMI is directly related to water intake because water is essential in the digestive process, and necessary for nutrient absorption, elimination of undigestible materials and waste products, such as heat (NRC 2001; Langhans *et al*., 1995).

The behavior observed for urination frequency was similar to what was observed for water intake frequency, because both act in the thermoregulation process of the animals (Mader and Davis, 2004) (Fig. 3C and D). The animals were subjected to the same environmental and nutritional conditions, so it is understandable that there were no changes in water intake frequency and, consequently, urination frequency. As there was no difference in DMI among treatments, the frequency of defecation was not affected, which was concentrated from 6am-12pm and 12pm-6pm, which were times of higher DMI, due to the longer grazing time by the animals (Fig. 3E).

The extreme weather conditions of solar radiation (2,467 µmol.m^{-2}.s^{-1}), and air temperature above 36.3°C associated with low air relative humidity (42.26%) observed between 12pm and 6pm hours affected the physiological parameters of the sheep (Fig. 1). Eustáquio Filho *et al*. (2011) studied Santa Inês lambs in climatic chamber and suggested that the best comfort zone temperature for sheep is 25°C and air relative humidity of 65%. The information above indicates that between 12pm and 2pm, which was the time of the physiological evaluations, the sheep were outside the thermal comfort zone and inevitably went through physiological adjustments to maintain the body temperature within an acceptable limit (Baêta and Souza, 2012). The average body surface temperature (BST) increment of 10 ºC in sheep from 6am to 8am affected the respiratory rate (RR), which showed an average value of 122 movements.minute^{-1}, being higher by 44.67% in the afternoon period (Fig. 4). Thus, that value characterizes high stress, close to 120 movements.minute^{-1} according to Silanikove (2000), but the animal grazing was not affected by the possible heat stress, because at that time, between 12pm and 2pm hours, there was intense grazing activity.

The heart rate (HR) followed the same response pattern as RR, showing an increase of 31% in the average of the treatments in the afternoon period. This variable is influenced by ambient temperature and is considered an indicator of heat stress (Mendes *et al*., 2013). The increase in HR is related to greater muscle activity to control RR or, due to the increase in peripheral vascularization, as an attempt to dissipate heat through the skin (Façanha *et al*., 2019). According to Queiroz *et al*. (2015), the normal range for this variable is 70 to 80 beats.minute^{-1}, indicating thermal comfort. In this context, the animals showed a slight change in HR in the afternoon, showing an average of 109 beats.minute^{-1} and, being normal in the morning, with an average of 74 beats.minute^{-1}.

Additionally, RT is a variable of extreme relevance because its increase is indicative of caloric increment, associated to endogenous heat of the diet, according to Ferreira *et al*. (2021), which is related to reduced animal performance when the rectal temperature is increased by 1.0ºC (McDowell *et al.*, 1974). The variation observed for RT in this study remained within the normal range (38.84 - 39.75ºC) (Bezerra *et al*., 2011). Thus, the results suggest that Santa Inês sheep are more adapted to pastoral systems and efficient in the elimination of heat, portraying their adaptability to semi-arid conditions and confirming the hypothesis that the physiological changes observed were due to environmental conditions and not to the management adopted.

CONCLUSIONS

Fertilization with *in natura* castor bean cake influences the structural characteristics of irrigated Tamani grass, reducing the productive rates of pasture when compared to mineral nitrogen fertilization. The use of detoxified castor bean cake as a substitute for soybean meal does not alter the behavioral activities and physiological parameters of sheep. In intensive pasture management, castor bean cake is more useful when detoxified and used as a supplement than *in natura* and used as organic fertilizer.

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