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Communication

[*Comunicação*]

Impact of diets containing spineless cactus associated with lipid sources or levels of urea on physiological parameters of lambs: preliminary studies

[*Impacto de dietas contendo palma forrageira associada a fontes lipídicas ou níveis de ureia sobre parâmetros fisiológicos de cordeiros: estudos preliminares*]

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Spineless cactus is a crop forage that stands out as animal feed in the tropics, being considered an energetic feed due to the high levels of non-fibrous carbohydrates (Pessoa *et al*., 2020). According to Silva *et al*. (2021), diets with high proportion of roughages, with high fiber content, promote greater caloric increase in the digestive process. In this sense, it is possible that the use of spineless cactus as part of the roughage can reduce the possibility of undesirable changes in the physiological parameters of animals, may reflect positively on animal welfare.

Several studies have focused mainly on the study of performance of sheep fed spineless cactus, thus not focusing on other aspects such as the interaction between nutrition and animal physiology. To date, there are no records of research that evaluated the combination between spineless cactus with lipid-rich alternative feeds or levels of urea on thermoregulatory responses of feedlot lambs in Northeast region of Brazil.

Impacts of thermal stress on animals have caused significant damage in lamb feedlot in tropical environments. According to Cabral *et al*. (2022), in regions with high temperatures, the animals intensify their physiological mechanisms for maintaining the thermal comfort and body homeostasis. However, it is important to point out that, in addition to the stress caused by the climatic variables of the environment in which the animals are raised, the level of ingestion and the nature of the feeds also affect the production of endogenous heat by ruminants, with consequent variation in physiological parameters.

The use of lipid sources in sheep feeding may have the objective of increasing energy density of diet without the need to increase fermentable carbohydrates in the rumen, which have a great influence on endogenous heat production. According to Burin (2016), lipids do not undergo the process of rumen fermentation and, therefore, do not produce endogenous heat. Thus, they can be added to the diet as a nutritional and thermal comfort strategy. Furthermore, the replacement of true protein sources by nonprotein nitrogen, such as urea, is carried out to reduce costs. The combination of urea and feeds rich in soluble carbohydrates of rapid availability in the rumen, such as spineless cactus, is fundamental so that there is adequate synchronization between energy and nitrogen availability (Pereira *et al*., 2020).

It was hypothesized that diets containing spineless cactus with grass hay and lipid sources or different levels of urea do not impair the functioning of sheep homeostatic mechanisms. Therefore, the aim of this study was to evaluate the physiological responses of feedlot lambs fed with lipid sources or increasing levels of urea associated with spineless cactus and Tifton-85 hay.

The experiments were approved by the Committee of Ethics in the Use of Animals,

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Federal Rural University of Pernambuco (UFRPE) (protocol numbers 9626051120 and 142/2018, for trials 1 and 2, respectively). The experimental studies were conducted at the Department of Animal Science of the UFRPE, Recife, Pernambuco, Brazil, located in the following geographical coordinates: latitude 8º04'03"S and longitude 34º55'00"W.

In experiment 1, twenty-four non-castrated male lambs, undefined breed, with a mean age of six months and mean initial body weight of 22.0±1.11 kg were distributed in a completely randomized design, with three treatments and eight replications, and housed in individual pens, with a water fountain and a feeder. In the pre-experimental period, all animals were weighed, identified, vaccinated against clostridium (Covexin 9®, São Paulo, SP, Brazil) and submitted to control of parasites, with doramectin 1% (Dectomax®, Guarulhos, SP, Brazil). The experimental period lasted 89 days of the feedlot, with 30 days of the adaptation to the diets and installations, and 59 days for data and sample collection.

The treatments consisted of three diets isoprotein with orelha de elefante mexicana (OEM) spineless cactus (*Opuntia stricta* [Haw]. Haw.), Tifton-85 hay (*Cynodon* spp.), ground corn (*Zea mays* L.), soybean meal (*Glycine max* L.), and mineral mix, combined with different lipid sources (cottonseed - CS: *Gossypium herbaceum* L.; extra-fat whole corn germ - EFWCG; or coconut by-product - CB: *Cocos nucifera* L.).

The diets were formulated to provide weight gain of 200 g/day (Nutrient…, 2007) and the roughage:concentrate ratio was 60:40 (Table 1).

Table 1. Ingredients proportion and chemical composition of the experimental diets

First experiment			
Ingredients (g/kg)	Diets		
	Cottonseed	EFWCG[a]	CB[b]
Tifton-85 hay	300	300	300
OEM[c] spineless cactus	300	300	300
Cottonseed	255	0	0
EFWCG[a]	0	130	0
CB[b]	0	0	65
Ground corn	65	170	225
Soybean meal	75	95	105
Mineral mix[†]	5	5	5
Diet composition (g/kg dry matter, unless stated)			
Dry matter (g/kg FM[d])	650.8	642.3	642.1
Ash	72.7	69.3	69.4
Crude protein	129.8	124.9	124.2
Ether extract	60	60.4	50.3
$_{ap}$NDF[e]	356.8	309.8	293.7
ADF[f]	246.7	176.2	178.9

[a] extra-fat whole corn germ, [b] coconut by-product, [c] orelha de elefante mexicana spineless cactus, [d] fresh matter, [e] neutral detergent fiber determined using heat-stable α-amylase and corrected for ash and protein, [f] acid detergent fiber. [†] Nutrients/kg of product: Ca = 127 g (min.); Ca = 228 (max.); P = 65 g (min.); S = 20 g (min.); Na = 162 g (min.); Co = 40 mg (min.); Cu = 200 mg (min.); I = 71 mg (min.); Mn = 1350 mg (min.); Se = 20 mg (min.); Zn = 1900 mg (min.); F = 765 mg (max.).

The animals were fed a total mixed ration (TMR), *ad libitum*, twice daily (08h00 and 15h00), and the amount of feed offered, and the leftovers were weighed daily to measure voluntary intake, guaranteeing leftovers of approximately 10% of the total dry matter (DM) offered. At the end of the experimental period of trial, the lambs were weighed.

Data were analyzed in completely randomized design in sub-divided plot scheme, allocating the treatment effect (diets) in the plots and in the subplots the effect of the evaluation shift (morning and afternoon). The data were submitted to analysis of variance (ANOVA) and the averages were compared by Tukey's test at 5% probability, using SAS statistical software (Statistical Analysis Systems, version 9.2).

In experiment 2, a total of forty non-castrated male Santa Inês lambs, approximately six months old and initial body weight of 22.2±2.1 kg were distributed in a completely randomized design, with four dietary treatments and ten replications. All the animals were accommodated in individual stalls, equipped with feeders and drinkers. Before starting the experiment, the lambs were weighed, identified, vaccinated against clostridium (Poli-Star, Vallée®, MSD Animal Health, Brazil), treated against ecto- and endoparasites (Closantel Sodium 10%; Hipra Saúde Animal®, Porto Alegre, Brazil), and supplemented with amino acids, minerals, and vitamins (organic modifier; Bravet® Laboratory, Rio de Janeiro, Brazil). The trial period involved 75 days (15 days of adaptation to the facilities and management and 60 days of the data and sample collection).

Treatments consisted of four diets with replacement of soybean meal by increasing levels of urea (0, 7.3, 14.6 and 21.9g/kg dry matter) associated with spineless cactus (*Opuntia stricta* [Haw]. Haw.), Tifton-85 hay, ground corn, soybean meal, and mineral mix. The urea used was mixed with sulfur flower in a ratio of 18:1.

The diets were isonitrogenous and formulated to meet the nutritional requirements of lambs with 30 kg of body weight and average daily gain of 250g (Nutrient…, 2007). The roughage:concentrate ratio was 70:30 (Table 2).

Table 2. Ingredients proportion and chemical composition of the experimental diets

Second experiment				
Ingredients (g/kg)	Levels of urea (g/kg dry matter)			
	0	7.3	14.6	21.9
OEM[a] spineless cactus	400	400	400	400
Tifton-85 hay	300	300	300	300
Ground corn	131.6	174.3	217.2	260.2
Soybean meal	150.7	100.6	50.4	0
Urea + sulfur flower (18:1)	0	7.3	14.6	21.9
Mineral mix[†]	17.8	17.8	17.9	17.9
Diet composition (g/kg dry matter, unless stated)				
Dry matter (g/kg FM[b])	239.09	239.08	239.08	239.07
Organic matter	906.7	909.4	912.1	914.8
Crude protein	142.5	142.7	142.9	143.2
Ether extract	19.8	20.5	21.2	21.9
$_{ap}$NDF[c]	334.9	331.3	327.7	324.1
Non-fibrous carbohydrates	409.4	426.5	443.6	460.7

[a] orelha de elefante mexicana spineless cactus, [b] fresh matter, [c] neutral detergent fiber determined using heat-stable α-amylase and corrected for ash and protein. [†] Nutrients/kg of product: Ca = 110 g (min.); Ca = 135 (max.); P = 87 g (min.); S = 18 g (min.); Na = 147 g (min.); Co = 15 mg (min.); Cu = 590 mg (min.); Cr = 20 mg (min.); I = 50 mg (min.); Mn = 2000 mg (min.); Mo = 300 mg (min.); Se = 20 mg (min.); Zn = 3800 mg (min.); F = 870 mg (max.).

The diets were provided *ad libitum* as a TMR, twice a day, at 08h00 and 16h00. The amount of feed supplied was corrected daily to maintaining approximately 10% of leftovers (DM basis). Daily DM intake was calculated as the difference between the total DM of feed offered and the DM in leftovers. The variables studied were interpreted through regression analysis performed in the Statistical Analysis System (Statistical…, 2009).

In both experiments, the lambs were maintained in a covered shed with constant illumination, housed in individual pens (1.00m x 1.20m) with a suspended slatted floor. The material used in the roof was fiber cement. A few minutes before each feeding, the Tifton-85 hay and spineless cactus were processing in forage machine (Tifton-85 hay was cut to 8 mm using Nogueira® Model PN Plus 2000, Brazil; and spineless cactus were cut to 25mm using MC1n Laboremus®, Campina Grande, Brazil). Weekly samples of the supplied feed and leftovers were collected for chemical analyses. Clean and fresh water was offered *ad libitum* during the experiments.

The diet ingredients and leftovers were dried in a forced ventilation oven at 55°C for 72h, ground in a Wiley mill with a 1 mm sieve and submitted to chemical analysis. DM, ash, crude protein (CP), and ether extract (EE) were determined according to AOAC (Official…, 1990). Neutral detergent fiber (NDF) and acid detergent fiber (ADF) were determined according to the methodology of Van Soest *et al*. (1991) modified by Detmann *et al*. (2012). Non-fibrous carbohydrates (NFC) were estimated using the equation recommended by Detmann and Valadares Filho (2010).

The environment was monitored with a black globe thermometer installed 1.20m approximately the floor. The black globe temperature was measured using a thermometer (mercury column) inserted into a black globe. A thermometric shelter was used, installed inside the sheds, also 1.20 m above the floor, containing a digital thermohygrometer for obtaining internal and external temperature. Dry bulb and wet bulb thermometers were utilized together considering relative humidity and dew point. The readings were performed twice a day (08h00 and 15h00), at times close to the animals' feeding. Incident rainfall data at the site and experimental months were recorded using a pluviometer installed in the proximity of the feedlot sheds. The records were made shortly after the occurrence of rain.

The climatological data obtained or estimated were air temperature (Ta), relative humidity (RH), temperature and humidity index (THI), black globe temperature (BGT), dew point temperature (DPT), and black globe temperature and humidity index (BGTHI). The equation proposed by Igono *et al*. (1992) was used to calculate the comfort index (THI). BGTHI was measured according to Buffington *et al*. (1981).

In experiment 1, the parameters studied were heart rate (HT), respiratory rate (RR), rectal temperature (RT), average skin temperature (ST), and forehead (FST), neck (NST), loin (LST) and shin (SST) surface temperatures (then, the mean skin temperature was calculated), evaluated during thirty days after the introduction of the tested diets, in the morning (between 8h00 and 9h00) and afternoon (between 15h00 and 16h00). In experiment 2, the variables HT, RR and RT were evaluated four hours after the morning feeding, during the last week of the trial period.

In both experiments, HR (in beats per minute) was obtained using stethoscope positioned between the third and fourth space left intercostal for fifteen seconds. This was later multiplied by four to calculate the number of beats per minute. The respiratory rate (movements per minute; mpm) was obtained through visual evaluation, through direct observation of the movements of the left flank for a period of fifteen seconds. To obtain the total number of respiratory movements per minute, the values were multiplied by four. Rectal temperature (°C) was evaluated using a digital veterinary clinical thermometer, introduced into the animal's rectum, until the sounder was triggered. Only in experiment 1, a laser-sight infrared thermometer (Model AK 32) was used to measure the temperatures of the forehead, neck, loin and shin surfaces. Then, the mean skin temperature was calculated.

In experiment 1 it was observed that the average ambient temperature and THI in the afternoon were higher than the average obtained in the morning (+1.48ºC and +1.0, respectively). However, the variables RH, BGT, DPT, BGTHI and precipitation showed higher averages in the morning shift (Table 3).

Table 3. Mean values of meteorological parameters and thermal comfort index during experimental shifts

First experiment							
Shift	Air temperature (ºC)	Relative humidity (%)	THI[a]	BGT[b] (ºC)	DPT[c] (ºC)	BGTHI[d]	Precipitation (mm)
Morning	28.82	72.31	79.97	31.25	23.20	81.10	53.25
Afternoon	30.30	64.66	80.97	30.70	22.70	80.37	17.97
Mean	29.56	68.48	80.47	30.97	22.95	80.73	180.94*

[a] temperature and humidity index, [b] black globe temperature, [c] dew point temperature, [d] black globe temperature and humidity index, * accumulated precipitation during all the experimental period.

Average air temperature observed during the morning and the afternoon (Tab. 3) were above 25°C, value considered ideal for sheep (Furtado *et al*., 2017). On the other hand, according to Baêta and Souza (2010), the average air temperature recorded in the morning shift remained within the thermal comfort zone for this species (between 15ºC and 30ºC). However, in the afternoon shift, the temperature exceeded the maximum limit of the thermoneutrality zone (30ºC) and they were below the upper critical temperature (35°C) (Furtado *et al*., 2017). These values indicate that, in the afternoon shift, the lambs could be subject to thermal stress. Similarly, in experiment 2, the ambient temperature (30.6ºC; Table 5) was above the thermal comfort range for sheep.

The mean of RH observed in the morning (72.31%) was above the levels considered ideal by Eustáquio Filho *et al*. (2011) (50-70%). In contrast, the value of this climatic variable was lower in the environment to which the lambs of experiment 2 were submitted and was within the normal variation range (Table 5). The highest air temperature and the lower RH observed in the afternoon shift are related to the lowest recorded rainfall, compared to the morning shift (Table 3).

In addition, in both studies, THI indicated no heat stress. According to Neves *et al*. (2009), THI below 82 characterizes the absence of heat stress in sheep. However, the THI found (Tab. 3) is considered alert for ruminants. According to Neiva *et al*. (2004), for sheep, THI between 74 and 81 represents a thermal alert situation. Heat stress in sheep increases as the relative humidity and ambient temperature exceed the thermal comfort zone (Oliveira *et al*., 2020).

A slightly higher BGTHI was observed in the morning shift (experiment 1). However, in both shifts and experiments, these values signal danger (79 to 84) (Baêta and Souza, 2010). In heat-stressful environments, thermoregulatory mechanisms of heat loss are triggered, which can reduce the productive and reproductive performance of animals. On the other hand, Andrade (2006) stated that an ITGU value of 85.1 cannot be classified as dangerous for Santa Inês sheep due to the high degree of adaptability of these animals to the climatic conditions of semi-arid regions. According to Pereira *et al*. (2022), Santa Inês lambs have high thermoregulatory efficiency under conditions of heat stress in the feedlot.

No significant interaction was observed between diet and shift (P>0.05). Physiological responses HR, RR, RT and surface temperatures in different body regions of lambs were not affected by the diets evaluated (P>0.05). On the other hand, HR, RT and SST in afternoon shift was higher than in the morning shift (P<0.05), with differences of 10.5 beat/min, and 0.2ºC and 1.05ºC, respectively (Table 4).

The superiority of HR and RT in the afternoon shift (experiment 1) is associated with higher ambient temperature, which exceeds the thermal comfort zone for sheep. These results are consistent with those reported by Furtado *et al*. (2017), when evaluating the physiological parameters in Santa Inês and crossbreed ewes in Brazilian semiarid. According to these authors, RT, ST, RR and HR of the sheep were higher in the afternoon.

Table 4. Productive and physiological parameters of lambs fed lipid sources associated with spineless cactus and Tifton-85 hay, in different shifts

Parameters	*First experiment*								
	Diets (D)			Shifts (S)			P-Value		
	Cottonseed	EFWCG[a]	CB[b]	Morning	Afternoon	CV (%)[#]	D	S	D*S
HR[c] (beat/min.[**])	138.80	137.44	140.40	133.75b	144.03a	11.47	0.870	0.031	0.697
RR[d] (mpm[***])	59.27	54.40	57.52	54.53	59.60	20.45	0.494	0.140	0.427
RT[e] (ºC)	39.08	39.05	39.11	38.98b	39.18a	0.76	0.856	0.023	0.721
ST[f] (ºC)	31.61	31.33	31.50	31.35	31.60	2.60	0.607	0.301	0.637
FST[g] (ºC)	32.11	31.72	31.85	31.90	31.89	2.42	0.358	0.933	0.645
NST[h] (ºC)	32.26	32.12	32.34	32.04	32.44	4.06	0.889	0.285	0.970
LST[i] (ºC)	31.54	31.47	31.34	31.68	31.23	3.85	0.899	0.204	0.553
SST[j](ºC)	30.55	29.99	30.44	29.80b	30.85a	2.77	0.145	0.001	0.624

[a] extra-fat whole corn germ, [b] coconut by-product, [c] heart rate, [d] respiratory rate, [e] rectal temperature, [f] skin temperature, [g] forehead surface temperature, [h] neck surface temperature, [i] loin surface temperature, [j] shin surface temperature; [#] Coefficient of variation; [**] beats per minute; [***] movements per minute. Averages in rows followed by different letters are statistically different by the Tukey's test at 5% probability.

Regardless of the shift, HR values were above the physiological standard, which should range from 70 to 110 heart beats per minute for animals in their thermoneutral zone (Kolb, 1981). RT varied in this study between 38.98°C and 39.18°C, which is within the limits of homeothermy. The normal physiological range of RT for sheep is between 38.3°C and 39.9°C (Robertshaw, 2006). This parameter signals that the physiological mechanisms (heart and respiratory rates, for example) were efficient in releasing heat and keeping the body temperature within the normal range.

The RR of animals fed all diets and in both shifts were above the normal range cited by Ribeiro *et al*. (2008), which is 20 to 36 mpm, indicating that the sheep used this mechanism to maintain homeostasis. However, the average RR of experimental animals (57.06 mpm) indicates, in ruminants, low stress (Silanikove, 2000). Small ruminants are influenced by climate effects, showing changes in RR, but with RT within the normal range (Cabral *et al*., 2022).

Only the skin surface temperature varied between shifts, being higher in the afternoon (P=0.001). This variation was probably due to the lower thermal gradient between the surface of the animals and the air temperature, due to increase in ambient temperature in this shift, as well as by the peripheral vasodilation, which increases the blood flow to the surface (Miranda *et al*., 2018; Cardoso *et al*., 2021). Probably, the change in temperature only in the shin is related to the closer proximity of this part of the body to the floor, which radiates heat.

There was a dietary effect on NDF intake (305.25; 203.97 and 185.72 g/day for CS, EFWCG and CB treatments, respectively; P=0.001), which could change the caloric increment of lambs and affect the evaluated physiological parameters (Silva *et al*., 2021). However, this variation in fiber intake was not able to affect thermoregulatory responses. Additionally, according to Silva *et al*. (2021), the greater amount of water provided by diets containing spineless cactus can contribute to the thermoregulation of animals, which can mitigate the effects of the environment on the physiological variables of sheep. Despite changes in physiological variables as a function of shift, weight gain was not influenced (133±0.03; 149±0.03 and 118±0.04 g/day for CS, EFWCG and CB treatments, respectively; P=0.090).

Regarding experiment 2, table 5 shows the mean values of the meteorological parameters and thermal comfort indices during trial.

There was no effect of increasing levels of urea replacing soybean meal (P>0.05) on DM and NDF intake, HR, RR and RT of lambs (Table 6).

Table 5. Mean values of meteorological parameters and thermal comfort index during experimental period

Shift	Air temperature (ºC)	Relative humidity (%)	THI[a]	BGT[b] (ºC)	DPT[c] (ºC)	BGTHI[d]	Accumulated precipitation (mm)
Second experiment							
Morning[*]	30.6	59.0	80.09	31.67	23.4	81.17	30.03

[a] temperature and humidity index, [b] black globe temperature, [c] dew point temperature, [d] black globe temperature and humidity index. [*] Mean values obtained at the time of evaluation of physiological responses (four hours after morning feeding).

Table 6. Dry matter and fiber intakes, and physiological parameters of lambs fed levels of urea associated with spineless cactus and Tifton-85 hay

Parameters	Levels of urea (g/kg dry matter)				SEM[*]	P-value	
Second experiment	0	7.3	14.6	21.9		Linear	Quadratic
Dry matter intake (g/day)	1115.6	1178.7	1141.2	1176.7	15.91	0.272	0.628
NDF[a] intake (g/day)	349.6	360.3	335.0	347.2	5.61	0.472	0.938
Heart rate (beat/min.[**])	119.8	114.5	119.3	124.4	1.66	0.210	0.120
Respiratory rate (mpm[***])	103.8	99.3	109.6	111.7	2.21	0.087	0.449
Rectal temperature (ºC)	39.31	39.38	39.42	39.31	0.02	0.804	0.063

[*]standard error of the mean; [*] beats per minute; [***] movements per minute; [a] neutral detergent fiber.

The results obtained in experiment 2 showed that diets containing different levels of urea did not negatively affect the thermoregulation of the lambs, which is associated with the similarity between intake of DM (average of 1153.05g/day) and NDF (average of 348.02 g/day) by animals (Table 6). All values of HR can be considered above the physiological standard for sheep (Kolb, 1981).

In all groups, the RR was well above the ideal, reaching approximately 112 mpm with the use of 21.9g/kg DM of urea, which corresponds to three times more than the upper limit established by Ribeiro *et al*. (2008) (20 to 36 mpm). Sheep use the airways as a primary mechanism for body heat dissipation, which is a good physiological indicator of heat stress (Marai *et al*., 2007). Similar to what was observed in the sheep from experiment 1, the RT of lambs fed different levels of urea in substitution by soybean meal remained within the normal range for the species (Robertshaw, 2006).

Our results demonstrate that diets containing different lipid sources or increasing levels of urea associated with spineless cactus and Tifton-85 hay as roughages did not promote changes in the main physiological responses of feedlot lambs. However, the afternoon shift, with weather conditions less favorable to thermal comfort, impairs body homeostasis of lambs fed with lipid sources combined with spineless cactus and Tifton-85 hay.

Keywords: alternative feeds, cactus cladodes, small ruminants, heat stress, thermoregulation

RESUMO

Dois experimentos foram realizados para avaliar as respostas fisiológicas de cordeiros confinados, alimentados com palma forrageira (PF) e feno de Tifton-85 (FT) associados a fontes lipídicas ou a níveis crescentes de ureia. No primeiro, 24 cordeiros machos, sem raça definida, foram alimentados com três dietas contendo diferentes fontes lipídicas (caroço de algodão, gérmen de milho integral extragordo ou película de coco seco). No segundo, 40 cordeiros machos da raça Santa Inês foram distribuídos em quatro tratamentos: dietas com substituição do farelo de soja por níveis crescentes de ureia (0; 7,3; 14,6 e 21,9g/kg na matéria seca). No experimento 1, a frequência cardíaca (FC) e a respiratória (FR), a temperatura retal (TR) e a superficial (ºC), em diferentes regiões corporais dos cordeiros, não foram afetadas pelas dietas (P>0,05). Porém, FC, TR e temperatura da canela, no turno da tarde, *apresentaram aumento (+10,5 batimentos/minuto; +0,2ºC; e +1,05ºC, respectivamente) em relação ao turno da manhã (P<0,05). Para o experimento 2, não houve efeito dos níveis de ureia (P>0,05) sobre o consumo de matéria seca e de fibra, a FC, a FR e a TR dos cordeiros. Portanto, dietas contendo fontes lipídicas ou níveis de ureia associados à PF e à FT não prejudicam importantes parâmetros fisiológicos de cordeiros.*

Palavras-chave: *alimentos alternativos, cladódios de palma, pequenos ruminantes, estresse térmico, termorregulação*

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