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INGESTIVE BEHAVIOR OF SANTA INÊS SHEEP UNDER THERMONEUTRALITY AND THERMAL STRESS UPON CONSUMPTION OF SALINE WATER

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KEYWORDS

climatic chamber, feed intake, idleness, *Ovis aries*, salinity, water intake.

ABSTRACT

The consumption of saline water in semiarid regions is a recurrent situation that can affect the ingestive behavior of small ruminants. This study evaluated the ingestive behavior of Santa Inês sheep maintained in a climatic chamber under two air temperatures -25 °C within the thermal comfort zone (TCZ) and 32 °C above the TCZ - and consuming water with three levels of salinity - 2.0, 4.0, and 8.0 dS m^{-1}. The experimental design was completely randomized in 2 × 3 factorial schemes, comprising two air temperatures and drinking water with three levels of salinity, in six replicates (animals). Regardless of air temperature, the consumption of saline water did not affect feed and water intake by the sheep; however, there was a decrease in feed intake and an increase in water intake as air temperature increased. Feed and water intake; feeding, rumination, and idle times; defecation frequency; and fecal production were not affected ($P > 0.05$) by the water salinity levels, whereas reduction in feed intake, feeding time, and rumination time and an increase in water intake and idle time were observed as air temperature increased. In summary, increasing air temperature negatively affected the ingestive behavior of the animals; however, water with salinity up to 8.0 dS m^{-1} did not affect their ingestive behavior and hence could be used for drinking.

INTRODUCTION

Thermal stress causes several changes in animal physiology, including a reduction in feed efficiency and intake, as well as disturbances in water, protein, energy, and mineral balance, limiting animal reproduction (Marai et al., 2007). Moreover, thermal stress causes a reduction in the production efficiency of animals (Dantas et al., 2015; Queiroz et al., 2015) and alterations in their rectal and skin temperatures; respiratory and heart rates (Furtado et al., 2017; Torres et al., 2017); and levels of thyroid, cortisol, and biochemical parameters such as total protein, albumin, globulin, cholesterol, triglycerides, urea, glucose, and gamma-glutamyltransferase (Costa et al., 2015).

The difficulty in obtaining good-quality water has intensified the demand of saline water for small ruminants to drink (Moura et al., 2016; Mdletshe et al., 2017; Paiva et al., 2017; Albuquerque et al., 2020), which characterizes a recurrent scenario in arid and semiarid regions wherein water may have salt levels above the recommended levels for domestic animals (Manera et al., 2016; Melo et al., 2017). Sheep such as those of the Santa Inês breed have excellent water restriction capability (Silva et al., 2016) and can consume forages that are chemically composed of high salt concentrations (Fahmy et al., 2010; Khanum et al., 2010; Moreno et al., 2015) and water with high salt levels (Yousfi et al., 2016; Castro et al., 2017; Furtado et al., 2020). Araújo et al. (2010) showed that sheep could tolerate water with a salinity of up to 8.0 dS m^{-1} without ingestion, causing harm to the health of the animals.

Factors such as elevation or air temperature can cause behavioral changes in sheep, including reduced feed intake and increased water intake (Torres et al., 2017; Furtado et al., 2020). In addition, the consumption of saline water promotes changes in the efficiency of feeding, rumination, and chewing (Moura et al., 2016), leading to

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compromise of performance of animal production. Albuquerque et al. (2020) reported that saline water intake affects the performance, digestibility, and nitrogen and water balance of feedlot lambs. In their study, the salts in the water promoted an increase in water consumption, minerals absorption and water excretion (via urine and feces) but did not affect the consumption of dry matter, digestibility of nutrients, total excretion of feces, and the production efficiency of animals.

In the reviewed literature concerning native sheep ingesting saline water, specifically in hot environment, it was observed a need to deepen its understanding. Based on the hypothesis that different water salinity levels and air temperatures modify the ingestive behavior of sheep breeds, this study evaluated the ingestive behavior of Santa Inês sheep maintained under thermoneutral and thermal stress conditions, while consuming water with different levels of salinity.

MATERIAL AND METHODS

Climatic Chamber

The animals were placed in two climatic chambers, each with an area of 19.71 m², ceiling height of 2.38 m, and laminated steel sheets with a layer of polyurethane and interior lighting with fluorescent light. The cooling system used was a split type air conditioner (Samsung Digital) with a capacity of 30,000 BTUs, and electric resistance air heaters were used for heating. Adjacent to the climate chambers was a control room with a temperature and humidity control board.

For humidification and dehumidification, a commercial humidifier and a dehumidifier were used. The equipment was coupled to the Full Gauge Controls® MT-530 PLUS control system, configured by the SITRAD software, which was responsible for recording and storing ambient temperature (TA) and relative humidity from the air (UR). Data acquisition was performed through a thermistor (TA) and a humidistat (UR), both located in a permeable envelope and positioned at the height of the center of mass of the animals (±1.50 m).

Animals

The Animal Ethics Committee of the Federal University of Campina Grande, Paraiba- UFCG, Brazil, approved the research protocol (Protocol number 105/13). A total of 18 uncastrated male animals of the Santa Inês breed were used, with an average age of 5.0 ± 0.5 months and weight of 17.0 ± 2.5 kg. The animals were previously raised in extensive systems in the Brazilian semiarid region and housed in metal stalls with dimensions of 1.15 × 0.50 × 0.84 m for the length, width and height, respectively, equipped with feeders and drinkers, and with a perforated iron floor for passing feces and urine. The animals were vermifugated against endoparasites and ectoparasites and were housed in metabolic cages (1.20 × 0.50 m = 0.60 m²) inside the climatic chamber, provided with feeders and drinkers.

The animals were distributed in a completely randomized design in a 2 × 3 factorial scheme, corresponding to two air temperatures and drinking water with three levels of salinity, with six replicates (animals). The animals were subjected to various air temperatures, and the average air temperatures recorded in the climatic chamber were 25.19 ± 1.18 ºC within the thermal comfort zone (TCZ) and 32.43 ± 2.03 ºC above the TCZ; for each air temperature, the average relative air humidity’s were 60.38 ± 5.11 and 60.73% ± 3.33, respectively, and the wind speed was 1.0 ± 0.4 m s^1. The experimental procedure was carried out in two stages, consisting of 18 animals (18 of each breed) at temperatures of 25.19 and 32.43 °C and three levels of water salinity (2.0, 4.0, and 8.0 dS m^{-1}). A period of 15 d of adaptation to the controlled environment, management, and feeding and 5 d of data collection were adopted in each step. Between the end of the experiment at one temperature and the beginning of the next, the animals were given 5 d to recompose their physiological functions.

The animals were subjected to a temperature-controlled 23 h program, wherein lights were turned off at 18:00 and turned on at 6:00 on the following morning, promoting 12 h of continuous light and midnight darkness. The chamber was opened for 1 h in the morning for cleaning and collection of leftover food and feces.

Water consumption was ad libitum and supplied daily, and the consumption was quantified according to the daily total supplied (7 L) minus the leftovers in the 24-h period. Salinity levels in the water were increased by dissolving sodium chloride in water, the amount of which was determined by the electrical conductivity of the water (ECa), according to the equation proposed by Richards (1954) to achieve the levels of 2.0 dS m^{-1}; 4.0 dS m^{-1} and 8.0 dS m^{-1}.

$$Q\ NaCl\ (mg\ L^{-1}) = 640 \times ECa\ (dSm^{-1}) \tag{1}$$

in which,

Q NaCl - amount of NaCl,

ECa - electrical conductivity of water.

Using a portable digital conductivity meter (ITCD 1000 model Instrutemp brand), the conductivity of the water to be added with NaCl was measured. Given this result, NaCl was dissolved and mixed in water until the solution reached the desired electrical conductivity.

Ingestive behavior

The feed provided to the animals consisted of *Cynodon dactylon* (L.) Pers. hay (Tifton) (50.20 kg), corn bran (34.69 kg), soybean meal (13.32 kg), limestone (0.45 kg), and mineral supplement (1.34 kg) composed of, in nutrient per kg of supplement, vitamin A (135,000.00 IU), vitamin D3 (68,000.00 IU), vitamin E (450.00 IU), calcium (240.00 g), phosphorus (71.00 g;), potassium (28.20 g), sulfur (20.00 g), magnesium (20.00 g), copper (400.00 mg), cobalt (30.00 mg), chromium (10.00 mg), iron (2,500.00 mg), iodine (40.00 mg), manganese (1,350.00 mg), selenium (15.00 mg), zinc (1,700.00 mg), and maximum fluorine (710.00 mg). The solubility of phosphorus in citric acid was 2.0% (min), making a chemical composition of 90.8% dry matter, 13.5% crude protein, 1.4% ether extract, and 51.9% neutral detergent fiber.

Ingestive behavior parameters were evaluated at the end of each experimental phase (15th d). Behavioral activities such as: 1) feeding, which is defined as eating when feed is still in the mouth; 2) ruminating, which is defined as chewing regurgitated feed, either in standing or lying position; 3) staying idle, which is defined as standing without any movement or behavior; and 4) drinking, which is defined as swallowing water were recorded according to the methodology proposed by Costa et al. (2019), by instantaneous and continuous sampling, using the focal sampling method and sampling intervals of 5 min in a direct fashion, with continuous periods of 24 h from 8:00 AM to 8:00 PM. The number of times the animal defecated, urinated, and sought water was visually and continually observed for 24 h by strategically positioned trained observers in an alternation system so as not to promote changes in the routine of the animals. During data collection of the nocturnal observation of the animals, the environment was kept under artificial illumination.

Statistical analysis

The animals were distributed in an experimental crossover design in a split-plot scheme of 2 × 3 × 2 (two ranges of environmental air temperatures × three levels of water salinity x two periods), with the temperature ranges being the main portion and water salinity levels in the sub-portion.

The data were analyzed using the PROC MIXED procedure of the SAS 9.1.3 software, considering the animal effect within the period as random as well as the carryover effect between the two periods. The average temperatures when the F test was significant and the average salinity levels when significant were compared using the Tukey-Kramer test ($P < 0.05$). The data were analyzed according to the following model:

$$Y_{ikl} = \mu + P_i + A_k + S_l + AS_{kl} + e_{ikl} \quad (2)$$

in which,

Y_{ikl} - observed parameters;

μ - general average;

P_i - period effect (i);

A_k - fixed temperature effect (k);

S_l - fixed effect for water salinity levels (l);

A_{Skl} - interaction of temperature effects (k) and water salinity levels (l),

e_{ikl} - residual effect for the interaction of temperature (k) and water salinity levels (l).

RESULTS AND DISCUSSION

The interaction between the air temperature and water salinity levels significantly affected ($P < 0.05$) the feed or water ingestion by the animals. There was no significant difference ($P > 0.05$) in the daily feed intake and water among the water salinity levels tested; however, a decrease in feed intake and an increase in water intake ($P < 0.05$) was observed as air temperature increased (Table 1).

TABLE 1. Average and standard deviations of feed and water intake by sheep under different air temperatures and water salinity levels.

Air temperature (ºC)	Salinity levels (dS m^{-1})	Feed intake (kg)	Water intake (L)
25	2	0.94 ± 0.03 aA	1.86 ± 0.26 aB
	4	0.95 ± 0.03 aA	1.78 ± 0.47 aB
	8	0.93 ± 0.06 aA	1.98 ± 0.28 aB
32	2	0.74 ± 0.02 aB	2.81 ± 0.30 aA
	4	0.75 ± 0.02 aB	2.78 ± 0.21 aA
	8	0.75 ± 0.03 aB	2.86 ± 0.30 aA
P value			
Air temperature		<0.0001	<0.0001
Salinity		0.5689	0.5234
Air temperature x Salinity		0.0023	0.0003

^{A,b}The average temperatures followed by the same capital letter in the columns are statistically equal by the Tukey-Kramer test at 5% probability; ^{a,b} The averages of the salinity content in the water followed by the same lower case letter in the columns are statistically equal by the Tukey-Kramer test at 5%

There was a significant difference in the feeding time, rumination time, and idle time (P < 0.05), with the interaction between the air temperature and water salinity levels (Table 2), demonstrating that sheep could maintain their ingestive behavior when consuming saline water. There was a significant reduction (P < 0.05) in the time the animals spent in the activities of feeding and rumination with the increase in air temperature and an increase (P < 0.05) in idle time. During the 24 h of evaluation at the air temperatures of 25 and 32 °C, the animals spent more time in idle activity, i.e., 42% and 50%, followed by rumination (38% and 35%) and feeding (19% and 14%), respectively.

TABLE 2. Average feeding, rumination, and idle times of sheep under different temperatures and water salinity levels.

Air temperature (ºC)	Salinity levels (dS m^{-1})	Feeding (h day^{-1})	Rumination (h day^{-1})	Idle (h day^{-1})
25	2	4.67 ± 0.71aA	9.21 ± 0.72 aA	10.11 ± 1.30 aB
	4	4.33 ± 1.79 aA	9.47 ± 1.14 aA	10.19 ± 2.92 aB
	8	4.74 ± 0.63 aA	9.08 ± 1.41 aA	10.18 ± 1.99 aB
32	2	3.64 ± 1.46 aB	8.71 ± 0.94 aB	11.64 ± 1.64 aA
	4	2.58 ± 0.66 aB	8.69 ± 1.09 aB	12.72 ± 1.67 aA
	8	3.97 ± 0.83 aB	8.36 ± 3.08 aB	11.66 ± 3.62 aA
P value				
Air temperature		0.0023	0.0326	0.3687
Salinity levels		0.7891	0.6257	0.7854
Air temperature x Salinity levels		<0.0001	0.0035	<0.0001

^{A,b}The average temperatures followed by the same capital letter in the columns are statistically equal by the Tukey-Kramer test at 5% probability; ^{a,b} The averages of the salinity content in the water followed by the same lower case letter in the columns are statistically equal by the Tukey-Kramer test at 5%

The different salt concentrations in the water did not cause significant changes ($P > 0.05$) in the defecation frequency, urinary frequency, water seeking, fecal production, or urine production (Table 3). Such similarity in the analyzed parameters was correlated with the intake of feed and water. The increase in air temperature caused a significant increase ($P < 0.05$) in the number of times the animals defecated, urinated, and sought water but reduced their fecal and urinary production.

TABLE 3. Distribution of the punctual activities, namely defecation, urination, and drinking, in the number of times/sheep/day under different temperatures and water salinity levels.

Air temperature (ºC)	Salinity levels (dS m^{-1})	Punctual activities (times/sheep/day)			Production (kg)	
		Defecation	Urination	Water-seeking	Fecal	Urinary
25	2	22.00 ± 2.60 aB	19.80 ± 4.60aB	8.62 ± 3.00 aB	0.75 ± 0.36 aA	1.27 ± 0.14 aA
	4	21.00 ± 4.60 aB	20.00 ± 4.20aB	10.62 ± 2.50 aB	0.72 ± 0.10 aA	1.65 ± 0.27 aA
	8	21.00 ± 3.60 aB	19.00 ± 3.00aB	9.02 ± 4.00 aB	0.71 ± 0.11 aA	1.85 ± 0.15 aA
32	2	25.6 ± 1.5 aA	22.60 ± 2.5aA	12.80 ± 3.00 aA	0.66 ± 0.12 aB	0.88 ± 0.14 aB
	4	26.6 ± 2.3 aA	24.0 0 ± 3.50aA	13.33 ± 2.30 aA	0.64 ± 0.21 aB	0.80 ± 0.13 aB
	8	24.0 ± 4.1aA	22.60 ± 2.30aA	11.95 ± 4.00 aA	0.53 ± 0.17 aB	0.82 ± 0.09 aB
P value						
Air temperature		0.02365	0.00232	0.01457	0.03654	0.00258
Salinity		0.05247	0.06243	0.47852	0.47895	0.96877
Air temperature × Salinity		<0.0001	0.00002	<0.0001	0.02536	<0.0001

^{A,b}The average temperatures followed by the same capital letter in the columns are statistically equal by the Tukey-Kramer test at 5% probability; ^{a,b} The averages of the salinity content in the water followed by the same lower case letter in the columns are statistically equal by the Tukey-Kramer test at 5%

The elevation in water salinity levels at each temperature did not interfere with feed intake, demonstrating that water salinity did not affect the ruminal conditions or metabolism of the animals. Moura et al. (2016) found no difference in dry matter intake by sheep consuming water with different levels of salinity. On the contrary, Yirga et al. (2018) found a reduction in metabolizable energy intake in adult sheep with an increase in salt concentration in the water. Moreover, Fahmy et al. (2010) reported that the voluntary intake of grasses was not affected by the level of water salinity, demonstrating the adaptive capacity of the species to drink saline water (Araújo et al., 2010).

The decrease in feed intake (26%) at the highest temperature is a consequence of the thermal stress on the animals, inducing the voluntary reduction of feed intake as a mechanism to reduce the production of endogenous heat, such as that from ruminal fermentation (Lucena et al., 2013) and metabolism. Thermal stress can cause disorders in sheep metabolism, affecting their physiology (Dantas et al., 2015), for example, by elevating their respiratory rate, heart rate, and rectal and surface temperatures (Furtado et al., 2017; Torres et al., 2017), as a means to eliminate the excess endogenous heat.

The salt levels supplied did not affect the water intake; this may have occurred because of the capacity of the sheep to adapt to the intake of saline water (Castro et al., 2017; Mdletshe et al., 2017), as part of the electrolytes contained in the saline solution are excreted through urine, feces, sweat, saliva, and bile when ingested in excess. The kidneys regulate the concentration of the electrolyte through aldosterone levels; when these levels are high, this substance stimulates urine elimination with a large quantity of electrolytes.

Water intake was higher at 32 °C because of the need of the organism for cooling by conduction and replacing the water evaporated via the respiratory tract and skin. According to Araújo et al. (2010), water intake is higher when the animals are subjected to high air temperatures. Moreover, Paiva et al. (2017) did not observe alterations in the production, intake, or digestibility of nutrients in lactating goats consuming water with high salinity levels.

It was observed that idle time was prioritized by the animals at a temperature of 32 °C, increasing by 15.34% when compared to that at the temperature of 25 °C, with this long time related to the shorter time spent in feeding and rumination as well as the reduced movement of the animals as a way to save energy, leading to lower heat generation.

No significant differences were observed regarding salinity levels in any of the parameters under each temperature. Food consumption was not affected by consumption of water with different salinity levels; therefore, rumination and food intake did not change, both corroborating with the results reported by Moura et al. (2016); however, they did not record differences in the feeding time, rumination time, or idle time of Santa Inês sheep as a result of the increase in water salinity levels.

The shorter time dedicated to feeding and rumination at the highest temperature is associated with lower feed intake (Table 1). Consequently, a lower supply of nutrients and fibers reaches the rumen and other digestive compartments, and this reduction is a mechanism used to reduce endogenous heat production. Marai et al. (2007) reported that sheep exposed to high air temperatures have negative biological functions, ultimately compromising their productive efficiency and reproduction.

There may be an increase in the amount of urine and excretion of minerals with the increase in salt concentration in the drinking water, requiring higher water filtration in the kidneys. However, the water salinity levels used were not sufficient to alter urinary frequency and production. Albuquerque et al. (2020) reported that the increase in the concentration of salts in water promoted an increasing linear effect for water intake and total excretion of water via urine, but it did not affect the consumption of dry matter and total excretion of feces. The digestive tract does not fully absorb electrolytes originating from feeding; therefore, the excess amount is excreted (through urine, feces, sweat, saliva, and bile), but when they are absorbed in excess, they undergo little or no hepatic processing and are transferred to peripheral tissues to perform their hepatic functions (Cunningham, 2004).

There was a decrease in feed intake and, consequently, in dry matter intake at the highest temperature, which promoted a longer retention time in the digestive tract of the animals, leading to higher nutrient digestibility and an increase in water intake. The lower weight is associated with higher absorption of water in the large intestine, causing drier feces and, consequently, lower mass and higher defecation frequency. The decrease in urine weight can be explained by the elimination of water by other means of heat dissipation such as increased respiratory rate (Furtado et al., 2017; Torres et al., 2017) and sweating.

CONCLUSIONS

Supplying drinking water with a salinity of 8 dS m^{-1} to the sheep during feeding, ruminating, or idling can be an alternative for sheep in regions where water has a high level of salinity.

Sheep exposure to a temperature of 32 °C caused modifications in the indicators of ingestive behavior compared to the values obtained at a temperature of 25 °C, with a reduction in feed intake, an increase in water intake and idle time, and a decrease in rumination and feeding times.

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