

## Goats in a comfortable and stressed environment consuming saline water: performance, digestibility, nitrogen balance, and urinary mineral concentrations

[Caprinos em ambiente de conforto e estresse consumindo água salina: desempenho, digestibilidade, balanço de nitrogênio e concentrações minerais urinárias]

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### ABSTRACT

The objective was to evaluate the effect of water salinity and environmental temperature on the nutrient consumption, digestibility, nitrogen balance, and mineral excretion of creole goats. Thirty-six males with an average age of  $5.0 \pm 0.6$  months and an average weight of  $20.0 \pm 2.3$  kg were housed in metabolic cages. They are distributed in a completely randomized design, with a  $2 \times 3$  type crossover (2 temperatures ( $T_1 = 26 \pm 0.6^\circ\text{C}$  and  $T_2 = 32 \pm 1.2^\circ\text{C}$ ) and three levels of salinity (1.0, 6.0, and  $12.0 \text{ dS m}^{-1}$ ). The temperature influenced ( $P < 0.05$ ) the intake of water and ether (EE) extract, the digestibility of EE, organic matter and dry matter, and the concentrations of calcium and potassium in the urine of goats. There was no significant effect ( $P > 0.05$ ) of temperatures or water salinity levels; the animals consumed and retained averages of 10.31 and 4.19 g day<sup>-1</sup> of nitrogen in the body, respectively. The different water salinity levels influenced ( $P < 0.05$ ) water intake and increased the excretions of potassium and sodium in urine. Total solids levels ranging from 640 to 9,600 mg L<sup>-1</sup> in water for goats increase water consumption, as does urine potassium and sodium excretion in urine.

Keywords: climatic chamber, dry matter, nitrogen, saline water

### RESUMO

O objetivo deste estudo foi avaliar o efeito da salinidade da água e da temperatura ambiente sobre o consumo de nutrientes, a digestibilidade, o balanço de nitrogênio e a excreção mineral de caprinos crioulos. Trinta e seis machos, com idade média de  $5,0 \pm 0,6$  meses e peso médio de  $20,0 \pm 2,3$  kg, foram alojados em gaiolas metabólicas. O delineamento foi inteiramente ao acaso, com cruzamento do tipo  $2 \times 3$  (duas temperaturas ( $T_1 = 26 \pm 0,6^\circ\text{C}$  e  $T_2 = 32 \pm 1,2^\circ\text{C}$ ) e três níveis de salinidade (1,0, 6,0 e  $12,0 \text{ dS m}^{-1}$ ). A temperatura influenciou ( $P < 0,05$ ) a ingestão de água e o extrato etéreo (EE), a digestibilidade de EE, a matéria orgânica e a matéria seca, bem como as concentrações de cálcio e potássio na urina de caprinos. Não houve efeito significativo ( $P > 0,05$ ) das temperaturas ou dos níveis de salinidade da água; os animais consumiram e retiveram médias de 10,31 e 4,19 g dia<sup>-1</sup> de nitrogênio no corpo, respectivamente. Os diferentes níveis de salinidade da água influenciam ( $P < 0,05$ ) na ingestão de água e aumentam nas excreções urinárias de potássio e sódio; os teores de sólidos totais variando de 640 a 9.600 mg L<sup>-1</sup> na água para caprinos aumentam o consumo de água, assim como a excreção urinária de potássio e sódio.

Palavras-chave: câmara climática, matéria seca, nitrogênio, água salina.

### INTRODUCTION

Small ruminants are adapted to the conditions of arid and semi-arid regions, so the raising of goats and sheep becomes a relevant activity in these regions (Silva *et al.*, 2016). Environmental

factors and the genetics of creole animals will be preponderant in their productive and reproductive capacity (Miranda *et al.*, 2018, Ribeiro *et al.*, 2018; Cardoso *et al.*, 2021). In arid and semi-arid regions, animals are naturally subject to factors such as high temperatures,

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Submitted: June 13, 2022. Accepted: January 10, 2023.

scarcity of water, or unsafe drinking water, as they present high levels of salt (Cardoso *et al.*, 2021), associated with long periods of drought and poor food supply. These factors can negatively affect animal production systems, even those in which the raising of creole goats predominates, relatively well adapted, and tolerant to conditions in the region (Cardoso *et al.*, 2021).

In these regions, the water used for animal consumption comes from dams, rivers, muds, and wells, which are superficial sources, with high evaporation values and large water losses, it is common for animals to ingest water with high concentrations of salts (Cardoso *et al.*, 2021).

Different studies indicate that nutrient intake and digestibility can be affected by several factors, such as species or breed of animals (Moura *et al.*, 2016), frequency of intake, and chemical composition of the feed (Moura *et al.*, 2016; Albuquerque *et al.*, 2020), ambient temperature and water salinity levels (Cardoso *et al.*, 2021). In addition, the results also indicate that goats show significant adaptability to saline water intake (Paiva *et al.*, 2017; Furtado *et al.*, 2020; Cardoso *et al.*, 2021), but under penalty of high salt concentration in water can affect dry matter intake by small ruminants and modify mineral metabolism. Therefore, metabolic costs arising from excess salts ingested in drinking water can negatively contribute to the performance of animals and, consequently, for the efficiency of production systems.

According to Cardoso *et al.* (2021) levels of total solids ranging from 640 to 9,600 mg L<sup>-1</sup> in water for goats do not cause changes in its variables, ingestive behavior, and efficiency. At the studied stress temperature (32°C) the goats keep the rectal temperature relatively constant, increasing water intake and evaporative heat loss. The rumination activity in goats is sensitive to an ambient temperature of 32°C, which reinforces the importance of microclimate management in places where the animals are kept.

Given the exposure, the hypothesis is that there is an interaction of environmental temperature and water salinity factors in modifying the consumption and metabolism of nutrients in the studied goats. Thus, studies that combine the two

factors mentioned above are relevant to elucidate and even recommend the ideal level of salinity that should be used in the drinking water of goats in different thermal situations. This study aimed to evaluate the effect of water salinity and environmental temperature on the nutrient consumption, digestibility, nitrogen balance, and mineral excretion of creole goats.

## MATERIAL AND METHODS

The Animal Ethics Committee approved this study of the Federal University of Paraíba (UFPB) protocol n°. 6167/18.

The research was conducted in two climatic chambers at the Animal Bioclimatology Laboratory, Department of Zootecnics, Center for Agricultural Sciences - UFPB, in Areia, state of Paraíba, Brazil with latitude 6°57'48"s, longitude 35°41'30"w and an elevation of 618 m.

The animals were placed in two climatic chambers, each with an area of 19.71 m<sup>2</sup>, a ceiling height of 2.38m, made of laminated steel sheets with a layer of polyurethane, and interior lighting of fluorescent light. The cooling system used was SPLIT model (Samsung Digital) air conditioners with the capacity of 30,000 BTUs and the heating through electric resistance air heaters. Adjacent to the climate chambers is a control room with a temperature and humidity control board. For humidification (BRITANIA, Fresh 5l, Curitiba, Paraná, Brazil) and dehumidification (ARTEL, EA16MR110, Bento Gonçalves, Rio Grande do Sul, Brazil), a commercial humidifier and dehumidifier were used, such as equipment coupled to the Full Gauge Controls® MT-530 PLUS control system, configured by SITRAD Software, responsible for recording and storing air temperature (AT) and relative humidity (RH) data. Data acquisition was performed through a thermistor and a humidistat, located in a permeable envelope and positioned at the height of the animal's center of mass ( $\pm 1.50$ m).

Thirty-six male goats of native breeds to the Brazilian semiarid region. The non-castrated males had an average age of  $5.0 \pm 0.6$  months and an average weight of  $20.0 \pm 2.3$ kg, which were initially weighed, identified, and housed in metabolic cages ( $1.20 \times 0.50 = 0.60$ m<sup>2</sup>) inside the

climatic chamber, provided with feeders and drinking fountains.

The experimental design was a 2x3 type crossover (two temperatures and three levels of salinity). The two temperatures were: T1 = 26±0.6°C (thermoneutral) and T2 = 32±1.2°C (stress) and 3 levels salinity of water (1.0, 6.0 and 12.0 dS m<sup>-1</sup>). The animals were submitted to relative humidity and average wind speed. 67.6±5.0 % and 2.0±0.4 mS<sup>-1</sup>, respectively. The experimental procedure was carried out in two periods. For each period, 15 days of adaptation to the controlled environment, management, and feeding, and five days of data collection were adopted. Between the end of one temperature and the beginning of the next, the animals were given five days to recompose their physiological functions. The salinity level was maintained in the animal when the temperature changed.

At each stage, the animals were subjected to a temperature-controlled 23h:00 program, where the lights were turned off at 18h:00 and turned on at 06h:00 the following morning, aiming to subject the animals to a period of midnight continuous light and midnight dark. The chamber was opened in the morning for one hour to clean it and collect leftover feed and feces.

The diet was provided with a forage: concentrate ratio of 50:50, aiming for a weight gain of 250g/day (Nutrient..., 2007). The experimental diet was composed of ground corn, soybean meal, and a vitamin and mineral supplement (Table 1). Feed intake was ad libitum, and the amount of feed ingested to establish 20.0 % of leftovers was estimated, and each animal was given two portions of 0.400kg per day, totaling 0.800kg/day/ animal. The animals were fed at 07:30 am and 16:00 pm.

Tale 1. Percentage and bromatological composition of experimental diet

Ingredient (g kg <sup>-1</sup> D.M.)	Diet
Tifton hay ( <i>Cynodon dactylon</i> , (L) Pers)	500
Ground corn	310
Soybean meal	170
Mineral supplement <sup>1</sup>	10
Calcitic limestone	5
<i>Chemical composition</i>	
Dry matter, DM (g kg <sup>-1</sup> in fed)	888
Crude protein, CP (g kg <sup>-1</sup> DM)	154
Ether extract, EE (g kg <sup>-1</sup> DM)	31.3
Neutral detergent fiber, NDF (g kg <sup>-1</sup> DM)	489
Fiber in acid detergent. FAD (g kg <sup>-1</sup> DM)	249
Ash (g kg <sup>-1</sup> DM)	64.5
Metabolizable energy, ME (kcal kg <sup>-1</sup> DM)	2.48

<sup>1</sup> Composition of mineral supplement. per kg, P, 70g; Ca, 140g; Na, 148g; S, 12g; Mg, 1320mg; F, 700mg; Zn, 4.700mg; Mn, 3,690mg; Fe, 2,200mg; Co, 140mg; I, 61mg; Se, 15mg; sodium monensin, 100mg.

Water consumption was ad libitum, supplied daily, and consumption was quantified according to the daily total supplied (07 L) minus the leftovers in the 24 h period. The experimental treatments consisted of waters with different levels of total dissolved solids (TDS): 640; 4,800 and 9,600mg L<sup>-1</sup>, obtained using sodium chloride (NaCl), electrical conductor of 1, 6, and 12 dSm<sup>-1</sup>.

The solutions were prepared in four water tanks, utilizing a source of water from the Federal University of Paraiba's water-supply system, without chemical treatments, in which sodium chloride without iodine - commonly found in the

market was added to reach the electrical conductivity desired. The water's conductivities from each treatment were read daily with a conductivity meter manufactured by Digimed®, allowing for a difference of 5% from each treatment limit. During the entire experiment, samples of water from each treatment were collected weekly, conditioned in labeled plastic bags, and subsequently frozen until they were analyzed. The samples were sent to, Federal University of Campina Grande's laboratory, where chemical analyses for chlorides, calcium, magnesium, potassium, and sodium were conducted, and where the electrical conductivity

and pH of the collected waters were also measured (Table 2).

During the five last days of the experimental period (in each period), feces, orts, and ingredients composing the diets were sampled. The samples taken from the piles were immediately frozen (-15°C) in a refrigerator for

further analysis. These samples were later homogenized to obtain a composite sample of each animal, and then the material was pre-dried in a forced ventilation oven at 55°C for 72h and milled with Willey-type knives using a sieve screen with 1 mm mesh for leavings and ingredients, and 2mm mesh for feces.

Table 2. Mean values of the conductivity variables, sodium, chlorine, calcium, magnesium, potassium, and pH of the waters offered to experimental goats

Variables	Levels of salinity (dSm <sup>-1</sup> )		
	1,0	6,0	12,0
pH	6.20	6.40	6.60
Conductivity (µS Cm <sup>-1</sup> )	0.09	5.26	11.42
Calcium (mg L <sup>-1</sup> )	32.0	15.0	18.0
Magnesium (mg L <sup>-1</sup> )	35.0	30.0	30.0
Sodium (mg L <sup>-1</sup> )	0.63	62.7	137.4
Potassium (mg L <sup>-1</sup> )	7.0	7.0	7.0
Chlorine (mg L <sup>-1</sup> )	1.83	78.5	176.5

The samples were analyzed following the protocols described by the Association of Official Analytical Chemists-AOAC (Latimer and Horwitz, 2010) for DM (method 934.01), crude protein (CP, method 984.13), ether extract (EE, method 920.39), ash content (method 942.05), and neutral detergent fiber (NDF; method AOAC 973.18). The total carbohydrates (TC) content was estimated by using the equation proposed by Sniffen *et al.* (1992): TC = 100 - (% CP + % EE + %ash). Non-fibrous carbohydrate (NFC) was estimated by using the equation proposed by Mertens (1997): NFC = 100 - (%CP + %EE + %DM + %NDF).

Estimation of total digestible nutrients (TDN) was based on the equation described by Weiss (1999): TDN = CPD + EED × 2.25 + NFCD + NDFcpD. In this equation, CPD = (CP ingested - CP feces), EED = (EE ingested - EE feces), NFCD = (NFC ingested - NFC feces), and NDFcpD = (NDFcp ingested - NDFcp feces). To calculate metabolizable energy (ME) (kcal ME/kg DM), the digestible energy (DE) was initially calculated as the product between total digestive nutrient (TDN) content and the factor 4.409/100, considering the ME concentration of 82% of DE (Silva and Leão 1979).

For urine collection, 25mL of 1:1 of 50% (wt/wt) solution of 95% HCl and water was placed in the collection containers daily between 16 and 20 days of the experimental period to prevent

fermentation and ammonia losses in the urine by volatilization. The total volume of urine excreted per animal was measured at intervals of 24 hours, and an aliquot corresponding to 10% of the total volume per animal was stored at -15 °C for later composition analysis in the Laboratory of Metabolic and Nutritional Diseases in Ruminants of the Federal Rural University of Pernambuco.

The minerals quantified in the urine were: Calcium ref.90 (Cresolfleina Liquiform), Phosphorus ref.12 (U.V. Liquiform), Potassium ref. 125 (enzymatic), Sodium ref.125 (enzymatic), Magnesium ref.50 (Magon/xylitol blue), using Calibra H as a calibrator and Qualitrol 2H as a control, by using Lab-test Diagnostica kits, in an automatic biochemical analyzer Labmax 240 (Labtest Diagnostic SA®, Brasil). The N balance was calculated as N balance (%) = (N intake - fecal N - urine N) / N intake × 100.

The data were analyzed using the PROC MIXED procedure of the SAS 9.1.3 software, considering the animal effect as random and the carryover effect between the two periods. The means when significant were compared using the Tukey-Kramer test (P<0.05). According to the following model:  $W_{ijk} = \mu + \alpha_i + b_j + \gamma_k + b_{jk} + \varepsilon_{ijk}$ . Where, in = observed variable;  $\mu$  = general average;  $\alpha_i$  = period effect (i);  $b_j$  = fixed effect of environment (j);  $\gamma_k$  = fixed effect for water salinity (k);  $b_{jk}$  =

interaction of the effects of environment (j) and water salinity (k);  $\epsilon_{ijk}$  = residual effect for the interaction of environment (j) and water salinity (k) factors.

**RESULTS**

There was no effect ( $P>0.05$ ) of the interaction of factors (water temperature and salinity) on the studied variables of nutrient intake and digestibility, nitrogen balance, and mineral concentration in urine.

The consumption of water ( $P<0.001$ ) and ether extract ( $P<0.001$ ) changed significantly with increasing temperature. there is an increase of 22.59% in water consumption from the

temperature of 24 to 32°C and a decrease in EE consumption of 75% at the temperature of 32 °C (Table 3). Water consumption was also influenced ( $P<0.001$ ) by the level of salt in the water, there was a 17% increase in water consumption at the level of 12 compared to the 1 and 6 dS-1 salinity levels. The digestibility of EE ( $P<0.001$ ), OM ( $P=0.004$ ), and CP ( $P<0.001$ ) was influenced by the increase in temperature. OM and CP increased their concentrations at a temperature of 32 °C, while EE digestibility decreased (Table 3).

The nitrogen balance was not influenced ( $P>0.05$ ) by the temperature and salinity level in the water (Table 4).

Table 3. Intake and digestibility of nutrients from native goats at 26 and 32 °C, consuming water with different salt levels

Effect		Intake (kg day <sup>-1</sup> )							Digestibility (%)		
		DM	OM	CP	EE	NFC	TC	NDF	Water	DM	OM
Temperature (°C)	26	0.677	0.555	0.098	0.04	0.188	0.496	0.363	1.78	78.80	85.56
	32	0.621	0.494	0.097	0.02	0.190	0.495	0.387	1.83	81.50	89.05
Mean standard error		0.03	0.03	0.004	0.003	0.01	0.01	0.02	0.70	1.06	1.01
Salinity (dSm <sup>-1</sup> )	1	0.651	0.522	0.094	0.01	0.193	0.484	0.364	1.49	79.84	86.75
	6	0.665	0.541	0.098	0.02	0.185	0.500	0.379	1.52	81.79	87.83
	12	0.631	0.509	0.099	0.03	0.189	0.501	0.381	1.78	78.82	87.34
Mean standard error		0.03	0.03	0.003	0.003	0.02	0.02	0.001	0.70	1.88	0.92
<i>P-value</i>											
Salinity		0.662	0.694	0.620	0.496	0.929	0.727	0.586	0.344	0.286	0.502
Temperature		0.087	0.056	0.886	<.0001	0.895	0.941	0.125	0.379	0.018	0.091
Salinity*temperature		0.613	0.647	0.840	0.564	0.585	0.918	0.880	0.373	0.245	0.341

Total carbohydrates = TC; Non-fibrous carbohydrates = NFC; ether extract = EE; neutral detergent fiber = NDF; organic matter = OM; dry matter = DM; crude protein = CP; Means followed by different letters on the same line differ by the Tukey test ( $P<0.05$ )

Table 4. Nitrogen (N) balance in native goats consuming water at 26 and 32 °C with different salinity levels

Effect (g day <sup>-1</sup> )		N ingested	N feces	N urine	N retained
Temperature (°C)	26	10.54	3.79	2.69	4.06
	32	10.08	3.50	2.15	4.42
Mean standard error		0.46	0.32	0.64	0.86
Salinity (dSm <sup>-1</sup> )	1	9.89	3.68	2.77	4.30
	6	10.57	3.61	2.44	4.28
	12	10.48	3.64	2.06	4.36
Mean standard error		0.75	0.39	0.78	0.40
<i>P-value</i>					
Salinity		0.618	0.981	0.668	0.477
Temperature		0.458	0.380	0.405	0.413
Salinity*temperature		0.936	0.321	0.251	0.694

Means followed by different letters on the same line differ by the Tukey test ( $P<0.05$ )

The calcium ( $P=0.0031$ ) and potassium ( $P=0.001$ ) concentrations were influenced by temperature, there was a reduction in the concentration of these minerals in urine at a temperature of 32 °C (Table 5). Phosphorus ( $P=0.017$ ), potassium ( $P=0.019$ ), and sodium

( $P<0.001$ ) changed significantly with the inclusion of salt in water, it is observed that phosphorus decreased its concentration in urine, as potassium and sodium increased their concentrations in urine.

Table 5. The concentration of minerals in the urine of goats of the native breeds at temperatures of 26 and 32 °C consuming water with different salinity levels

Effect		Calcium (mmol L <sup>-1</sup> )	Potassium (mg L <sup>-1</sup> )	Magnesium (mmol L <sup>-1</sup> )	Sodium (mg L <sup>-1</sup> )	Phosphorus (mmol L <sup>-1</sup> )
Temperature (°C)	26	1.79	21.61	3.48	101.38	3.08
	32	1.16	17.56	3.64	111.40	1.89
Mean standard error		0.29	1.29	0.17	10.18	0.54
Salinity (dSm <sup>-1</sup> )	1	1.14	17.14	3.65	34.49	2.62
	6	1.73	20.89	3.52	125.10	3.19
	12	1.56	20.72	3.51	159.58	1.65
Mean standard error		0.36	1.58	0.30	12.46	0.66
<i>P-value</i>						
Salinity		0.266	0.054	0.874	<.0001	0.267
Temperature		0.042	0.007	0.504	0.496	0.129
Salinity*temperature		0.237	0.439	0.575	0.459	0.156

Means followed by different letters on the same line differ by the Tukey test ( $P<0.05$ )

## DISCUSSION

The water intake of goats can be affected by the concentration of minerals such as calcium, phosphorus, magnesium, and sulfur. Generally, an increase in these components is associated with a decrease in water intake and may also result in decreased DM intake (Nutrient..., 2007). In our study, water and DM intake by goats were not affected by salinity or water temperature.

Water with electrical conductivity from 8.0 to 11.0 dS m<sup>-1</sup> can be limiting for ruminants, including goats, and above 11 dS m<sup>-1</sup> can be of high risk for animals (Araújo *et al.*, 2010). Abdelsattar *et al.* (2020) stated that the ability of animals to tolerate saline water depends on the level of salinity and minerals in the water. The no change in water intake of the animals under study confirms the adaptability of goats to hot weather, where they use physiological and behavioral mechanisms to adapt to this type of environment (Aiura *et al.*, 2010; Miranda *et al.*, 2018). This adaptation can be better perceived by making the ratio between average water and DM intake at the temperatures evaluated, which was 2.75kg of water per kg of DM, being within the pre-established rate by the NRC (Nutrient...,

2007), which should be 2.87 liters of water/kg DM.

The animals exposed to a higher temperature spent more time idle, remaining homeothermic with less energy expenditure because the shorter time of rumination also contributed to reducing heat production (Cardoso *et al.*, 2021). Thus, we observed that the decreased consumption of ethereal extract by goats subjected to a higher temperature can be related to the lower need for production and dissipation of body heat (Wolp *et al.*, 2012). Animals kept in a high temperature environment need to eliminate latent body heat, such as respiration, peripheral vasodilation, and sweating (Furtado *et al.*, 2020). Ribeiro *et al.* (2018) mention that, regardless of breed, environmental variables trigger physiological and behavioral changes in goats to maintain their homeothermia.

The DM digestibility was reduced for animals that were exposed to a higher temperature. This fact can be attributed to the intake of saline water because the level of salinity of the water can interfere with the intake and digestibility of nutrients (Furtado *et al.*, 2021). Salt intake can alter the fermentation pattern in the rumen, affecting the acid-base balance. Thus, the animal

consumes more water to balance this effect, which can reduce the adhesion of bacteria to food particles in the rumen and increase the renewal rate of solid and liquid periods, thus reducing rumen digestion (Giger-Reverdin *et al.*, 2020).

Another answer for the adaptability of goats is that the nitrogen balance is not altered by the level of salinity of the water, nor by thermal stress. Normally, the nitrogen balance indicates protein metabolism and is an essential parameter in evaluating the diet and assessing whether the animal is in balance concerning nitrogen compounds (Nobre *et al.*, 2020). The protein intake by the study animals was not affected by the treatments, with an average of  $0.10 \text{ kg}^{-1} \text{ animal}^{-1} \text{ day}^{-1}$ , within the pre-established by the NRC (Nutrient..., 2007).

Renal function, as well as mineral balance, can be affected by the level of food and water intake (Maciel *et al.*, 2016). In the present study, the non-variation in the urinary excretion rate of minerals (magnesium, sodium, and phosphorus) at different temperatures is justified because the main route of excretion of these minerals is through feces, and their excretion in urine is very low.

However, the salinity of the water influenced the levels of urinary excretion of the sodium and potassium minerals. One of the reasons for the increase in this excretion is that the animal organism does not store sodium and its reserves are limited, so excess sodium is quickly excreted in the urine. Potassium is closely related to sodium, and sodium intake in the body is negatively correlated with potassium excretion (Dewhurst *et al.*, 1968), the lower the Na intake, the greater the K excretion. But this did not occur in the present study may be due to the amount of magnesium excreted being close to potassium. Therefore, saline water with higher salinity levels causes an elevation in the excretion of minerals.

### CONCLUSIONS

Total solids levels ranging from 640 to 9,600mg L<sup>-1</sup> in water for goats increase water consumption, as does urine potassium and sodium excretion. The nitrogen balance as well as the intake and digestibility of nutrients do not change with the consumption of salt water by

goats. Therefore, water with up to 9,600mg of TDS L<sup>-1</sup> can be strategically used in goat watering in semi-arid regions.

### ACKNOWLEDGEMENTS

The authors wish to thank the Brazilian National Council for Scientific and Technological Development (CNPq)

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