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Meat quality in ewes submitted to reduction in water supply

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ABSTRACT

The aim of this study was to evaluate the reduction in water supply (*ad libitum* intake - 100%, or 80%, 60% and 40% of *ad libitum* intake) on the proximate composition, physicochemical properties, mineral composition and fatty acid profile of ewe meat. Thirty-two crossbred Santa Inês ewe (n = 8 per treatment) were distributed in a randomized block design, receiving a diet consisting of elephant grass and concentrate (70:30). The experimental period lasted 63 days, preceded by 14 days of adaptation. Ewes were slaughtered at the end of experimental period, at a mean final body weight of 37.63 kg. Carcasses were cut lengthwise and the loin (*longissimus lumborum*) was taken of the left half carcass to evaluate the proximate composition, the physicochemical characteristics, the minerals content and fatty acid profile. The reduction in water supply resulted in a quadratic effect for resilience ($p < 0.05$), Lightness L* ($p < 0.05$), potassium ($p < 0.05$) and iron content ($p < 0.001$). There was a decreasing linear effect for magnesium content ($p < 0.05$), copper content ($p < 0.001$), C18:1n7t content ($p < 0.05$) and Σmonounsaturated content ($p < 0.05$). Crossbred Santa Inês ewe tolerate water restriction up to 40% voluntary intake without compromising the meat quality.

1. Introduction

In dryland regions, due to irregular rains, the supply of food and the scarcity of water for the animals, are considered problems to be faced in production systems (Souza et al., 2020). The growing water scarcity has attracted concerns from different segments of society, seeking solutions for the rational and sustainable use of this natural resource (Ibidhi et al., 2017; Araújo et al., 2019).

Water is intrinsically linked to all biochemical processes and promotes the homeostasis of the organism (Ponnampalam et al., 2016). Water scarcity for animal consumption has the consequences of reduced growth, well-being and health and increased stress, that is, it results in considerable negative impacts on productive and economic factors. These impacts are well known to livestock producers (Araújo, 2015). In small ruminants, water deprivation can lead to skin retraction, dry eyes, weight loss, low food intake, dry faeces and reduced urine excretion (Souza et al., 2022).

According to Silva et al. (2016), small ruminants have access to free or supplied water; water contained in the food; and metabolic water. The amount of water formed from oxidation in the body depends on the type of feedstuff metabolized, and the catabolism of 1 kg of fat, carbohydrate, and protein produces 1.1, 0.5, and 0.4 L of water, respectively. In addition, to support the low water availability, animals reduce food intake to decrease the metabolic rate, generating less heat in the digestive process (Santos et al., 2019). Thus, animals increase their ability to withstand water deficit.

In Semi-arid Region of Northeastern Brazil, sheep are typically exploited for meat production in an extensive rearing system, with little or no technology (Souza et al., 2019). In this context, the Santa Inês breed is identified as a promising alternative for the meat production, due to its adaptability, reproductive efficiency and low susceptibility to endo and ectoparasites (Pereira et al., 2017), playing an important role

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in protein production in dry climate areas.

In meat tissues, water is found in a large proportion (about 75%) and is related to the physical characteristics of meat, such as: drip loss, texture/firmness, pH and color (Ponnampalam et al., 2016). According to Hoekstra (2011) the estimated water footprint for sheep meat production is 6.10 L/kg meat. Thus, the study of water levels required by small ruminants, specifically by ewes in confinement, makes it possible to reduce the water footprint in meat production, justifying the need to assess the minimum amount of water required without changing the nutritional quality of sheep meat.

According to Barbour et al. (2005), although small ruminants in dryland regions can survive up to a week with little or no water, the deficiency of this nutrient negatively affects homeostasis, body weight, reproductive rate and disease resistance, in addition to the possibility of having negative impacts on the meat quality. D'Ambrosio et al., (2018) found that small ruminants that underwent a period of 8 days of water restriction showed a 72% increase in the level of circulating cortisol, which can affect the quality of the meat, reducing its tenderness. Already Santos et al., (2019) found that water restriction for a period of 3 days does not affect the tenderness of the meat. Several studies list the effect of different sexes, breeds, age at slaughter and feeding systems, as factors that alter the dynamics of meat quality (Cherif et al., 2018; Costa et al., 2018; Pinheiro et al., 2019). Although these aspects are relevant to scientific and socioeconomic knowledge, studies assessing the nutritional composition of meat from animals subjected to water stress in Brazilian semi-arid regions are lacking (Santos et al., 2019).

To the best of our knowledge, there are no studies on the effect of daily reductions in water supply on quality characteristics, mineral composition and fatty acid profile in ewe meat. Thus, studies are necessary to know and characterize the effect of water supply restriction on the quality of the meat produced in this scenario. In view of this, the aim of this study was to evaluate the influence of water supply on the proximate composition, physicochemical characteristics, mineral composition and fatty acid profile in meat of Santa Inês ewe.

2. Material and methods

2.1. Experimental site

The experiment was conducted in the Universidade Federal do Vale do São Francisco (UNIVASF), in Petrolina, Pernambuco, Brazil (9º19'28" South latitude, 40º33'34" West longitude, 393 m altitude). The climate is hot semiarid type, with rainy season (BSh), and average annual precipitation of 376 mm, unevenly distributed (Climate-Data, 2019). During the experimental period, the maximum and minimum temperatures were 33.83 and 24.56 °C respectively, and the relative humidity varied between 50.50% and 73.56%.

2.2. Ethical aspects

This research was approved by the Ethics Committee on the Use of Animals (CEUA) of the Federal University of the São Francisco Valley - UNIVASF, with protocol number 0002/241017.

2.3. Animals

Thirty-two Santa Inês ewes, multiparous, non-lactating, 2–3 ± 0.99 years old and 32.2 ± 7.4 kg body weight, were distributed in individual pens (1.00 × 1.20 m), provided with feeders and drinkers (10 L capacity). The experiment was a randomized block design with 4 treatments and 8 ewes per treatment. The initial bodyweight was used to define the blocks. The experimental period lasted 63 days, preceded by 14 days of adaptation. At the beginning of the adaptation, the animals were identified, weighed, treated against endo and ectoparasites and randomly allocated to the pens previously identified according to the treatments.

2.4. Treatments

The treatments consisted of different water supply levels: water *ad libitum* (Control – 100%), 80%; 60% and 40% supply of the control group. The water was supplied in buckets and weighed before supplied and weighed again 24-h later (leftovers). The water lost through evaporation was also considered when calculating the water supply of the treatments. This variable was estimated using buckets randomly spread across the experimental shed, with the same amount of water available for each treatment. The difference in weight was determined over 24-h. Water was offered once a day, at 09-h. The water supply was calculated daily according to the treatments, which resulted in average intake of 1790 g/day; 1410 g/day (78.77%); 1110 g/day (62.01%) and 730 g/day (40.78%). The water used for watering the animals came from the São Francisco River (Brazil). The water was transferred to the experiment site through pipes. Water samples were collected every 15 days for laboratory analysis (Table 1).

2.5. Experimental diet

The experimental diet was composed of fresh elephant grass cv. Cameron (*Pennisetum purpureum* Schun) and concentrate (corn meal, soybean meal, urea and mineral salt). Experimental diet was formulated with a forage: concentrate ratio 70:30 on a fresh matter basis, and balanced to allow a daily gain of 157 g/day, according to National Research Council (NRC, 2007). Samples of ingredients were taken to determine their chemical (Table 2) and mineral composition, and fatty acid profile (Table 3).

Food was offered daily at 09-h and 15-h and the leftovers were collected and weighed to determine the intake and adjust the dry matter intake (DMI) in order to allow 15% leftovers in the trough. During the experimental period the ewe's sheep had an average dry matter intake and average daily gain of 1156 ± 98 and 77 ± 105 g/day (*ad libitum* water intake - 100% treatment), 1113 ± 117 and 99 ± 105 g/day (treatment 80%), 1170 ± 108 and 65 ± 38 g/day (treatment 60%) and 1180 ± 114 and 100 ± 29 g/day (treatment 40%), respectively.

2.6. Slaughter and loin collection for analysis

Ewes were slaughtered at the end of the experimental period, at a mean final body weight of 37.63 kg, after fasting for 16-h, according RIISPOA (2017). The animals were previously stunned and immediately slaughtered cutting the main blood vessels in the neck. After skinning and evisceration, the carcasses were transferred to a cold room (4ºC; 24-h). Carcasses were cut lengthwise and the loin (*longissimus lumborum*) were removed between the 12th/13th ribs. The loin was dissected to remove subcutaneous fat (Corlett et al., 2021). The samples were individually wrapped in aluminum foil and stored at − 20 °C until laboratory analysis.

2.7. Proximate composition of ewe meat

Meat samples were thawed in a 8 °C for 12-h under refrigeration and ground in a food processor (Mallory, Oggi+, Rio de Janeiro, RJ, Brazil). The methodologies described by the AOAC (2016) were used to determine the moisture (protocol number 985.41), ash (protocol number 920.153) and protein (protocol number 928.08) contents. The lipid content was determined according to AOCS (2017).

2.8. Physicochemical characteristics of ewe meat

Meat samples were thawed in a 8 °C refrigerator for 12-h to perform the physicochemical analysis. The pH of the meat was obtained at 20 °C and measured with a portable digital pHmeter (Testo SE & Co. KGaA, Campinas, SP, Brazil), calibrated calibrated using buffer solutions of pH 4 and 7 according to the manufacturer's instructions. The pHmeter's

Table 1
Chemical and physical composition of water supplied to Santa Inês ewe during the experimental period.

Ca^{2+}	Mg^{2+}	Na^{+}	K^{+}	Cl^{-}	Bicarbonates	Sulfates	EC
mmol/L							ds/m
0.63 ± 0.03	0.74 ± 0.02	0.27 ± 0.02	0.18 ± 0.02	0.66 ± 0.03	0.32 ± 0.02	0.51 ± 0.01	0.08 ± 0.02
	Turbidity	TC	Color	Chlorine	TH	pH	
	NTU	MPN		mg/L			
	0.0 ± 0.0	0.0 ± 0.0	9.0 ± 0.4	1.5 ± 0.3	3.44 ± 0.04	6.98 ± 0.02	

Ca2 + = Exchangeable calcium; Mg2 + = Exchangeable magnesium; Na+ = Exchangeable sodium; K+ = Exchangeable potassium; Cl− = Chlorides; pH= Hydrogenionic potential; EC= Electrical conductivity; TH= Total Hardness CaCO3. TC= Thermotolerant Coliforms; pH = Hydrogenionic potential; NTU = Nephelometric Turbidity Units; MPN = most probable number.

Table 2
Chemical composition of the ingredients in the experimental diet.

Ingredient			g/kg DM	
Elephant grass			460	
Corn meal			381	
Soybean meal			132	
Livestock urea			7	
Mineral salt[a]			20	
Chemical composition (in g/kg DM)	Elephant grass	Corn meal	Soybean meal	Diet
Dry matter[a]	261.9	889.3	886.1	576.3
Mineral matter	105.2	12.9	64.8	61.9
Crude protein	105.5	89.9	487.4	149.1
Ether extract	28.7	45.1	19.0	32.9
Neutral detergent fiber	708.7	111.6	155.0	370.6
Acid detergent fiber	419.5	33.7	89.0	207.0
Total carbohydrates	830.5	859.9	428.0	715.3
Non-fiber carbohydrates	174.0	642.0	279.0	328.3
Total digestible nutrients	570.1	850.0	804.8	596.7

[a] Guarantee levels per kilogram of the product according to the manufacturer: Calcium (min.) 190 g; Phosphorus (min.) 75 g; Magnesium (min.) 10 g; Chlorine (min.) 218 g; Sulfur (min.) 70 g; Sodium (min.) 143 g; Copper (min.) 300 mg; Cobalt (min.) 405 mg; Iron (min.) 500 mg; Iodine (min.) 80 mg; Manganese (min.) 1100 mg; Selenium (min.) 30 mg; Zinc (min.) 4600 mg; Fluorine (max.) 0.87 g; Phosphorus (P) solubility in 2% citric acid (min): 95%. [a]in g/kg natural matter

Table 3
Mineral and fatty acid composition of ingredients and diet.

Mineral (mg/100 g)	Forage	Concentrate	Diet
Nitrogen	16.23	34.81	26.26
Phosphorus	4.51	11.67	8.37
Potassium	20.35	16.80	18.43
Calcium	6.77	15.83	11.66
Magnesium	3.21	1.95	2.52
Sodium	0.26	4.80	2.71
Sulfur	1.51	3.06	2.34
Boron	9.63	7.46	8.45
Copper	10.79	131.13	75.77
Iron	97.16	269.85	190.41
Manganese	47.96	79.51	64.99
Zinc	42.94	136.50	93.46
Fatty acid (g/100 g FA)			
C8:0	0.05	Nd	0.02
C10:0	0.04	Nd	0.02
C12:0	3.13	Nd	1.44
C13:0	0.60	Nd	0.27
C14:0	0.89	0.03	0.42
C14:1	0.75	Nd	0.34
C15:0	0.19	Nd	0.08
C16:0	27.75	15.47	21.12
C16:1	0.15	0.05	0.10
C17:0	0.21	0.04	0.12
C17:1	Nd	0.01	0.01
C18:0	4.34	0.45	2.24
C18:1n9c	6.27	34.30	21.41
C18:2n6–9c12c	16.96	47.76	33.59
C18:3n6–6c9c12c	3.03	0.28	1.55
C18:3n3–9c12c15c	33.09	1.24	15.89
C20:1n9	Nd	0.10	0.05
C22:0	1.90	0.10	0.93
C24:0	0.56	0.11	0.32

Nd= Not detected

skewer-type digital probe has been inserted at the center of the samples (AOAC, 2016). The pH was evaluated at three points within the muscle, adopting the average value of these three points.

The color analysis was conducted in a fresh cross-section of *longissimus lumborum* muscle cut sample and allowed to develop at temperatures between 6 and 7 °C for 40 min (Sousa et al., 2022). Meat color was analyzed using a colorimeter (Konica® Minolta CR-400, Osaka, Japan). The CIE system (Commission internationale de l'´eclairage) was used to read lightness (L^*; black/white), redness (a^*; green/red) and yellowness (b^*; blue/yellow), calibrated to a white standard, and having an aperture size of 8 mm, 10° for standard observation, and it was operated using open cone (Miltenburg et al., 1992). The aperture port had a glass cover, and the samples were measured using illuminant D65. This device was calibrated before each analysis with a white tile standard. After exposure of the samples to the atmosphere for 30 min for oxygenation of the myoglobin (Cañeque and Sañudo 2000), the color values were measured at three points on the surface of the samples (Silva et al., 2021). Chroma (c^*), hue angle (h^*) and the white intensity (W) were determined according to Pathare et al. (2013):

$$c^* = [(a^*2 + b^*2)^{l/2}]) \quad (1)$$

$$h^* = [\arctan (b^*/a^*)] \quad (2)$$

$$W = 100 - [(100 - L^*)^2 + a^{*2} + b^{*2}]^{1/2} \quad (3)$$

Water holding capacity (WHC) evaluated according to Honikel and Hamm (1994). Samples of 0.5 g loin were placed on filter paper, with an area of 10 × 10 cm^2 (Whatman #1), between two plexiglass plates. The set was pressed with a standard weight of 5 kg for 5 min. Then the samples were weighed again.

Cooking losses (CL, %) was determined using meat samples approximately 1.5 cm thick, 3.0 cm long, and 2.5 cm wide, following the methodology of AMSA (2015). The weight of the samples was recorded before and after cooking. The samples were cooked in a digital water bath (TECNAL, Piracicaba, SP, Brazil) at 100 °C, until the internal meat sample temperature reached 72 °C, measured using a copper-constant thermocouple equipped with a digital reader. CL were calculated by the difference between the initial weight and the final weight of the sample used to determine the loss due to cooking, expressed in %.

Shear force (SF, kgf/cm^2) were determined according to, using the same samples used to analyze the CL. After cooling to room temperature, the samples used in the CL determination were wrapped in aluminum foil and kept under refrigeration for 12-h at 8 °C. After this period, samples were evaluated on a texturometer (Texture Analyzer TA-XPLUS-30, Godalming, United Kingdom) fitted with a Warner–Bratzler-type shear blade (WBSF; Wheeler et al., 1995) with a thickness of 1.016 mm, a length of 3.05 mm, and a cutting speed of 200 mm/min, according to the standard procedure of the US Meat Animal Research Center

(Shackelford et al., 1999) The samples were arranged with the fibers oriented perpendicular to the blade.

The instrumental Texture Profile (Texture Profile Analysis - TPA) was determined using an texture analyzer (Texture Analyzer TA-XPLUS-30, Godalming, United Kingdom) with a 36-mm-diameter P/36 R metal probe. Data were measured using Texture Expert Exponent® software (Stable Micro Systems Ltd., Surrey, England) following the methodology proposed by Bourne (2002). The parameters evaluated were:

hardness, adhesiveness, springiness, cohesiveness, chewiness and resilience. The settings used were: constant speed of 3.0 mm/s (pre-test), 1.0 mm/s (test) and 3.0 mm/s (post-test), load cell capacity = 10 kg and force filter= 10 Hz (Bourne, 2002).

2.9. Mineral status of ewe meat

Nitrogen (N) was determined according to AOAC (2016). The concentrations of sodium (Na) and calcium (Ca) were analyzed using a flame spectrophotometer (AOCS, 2017). The contents of potassium (K) and magnesium (Mg) were determined according to the methodology of Harris and Lucy (2020). Phosphorus (P) was determined on a spectrophotometer, following a dilution of the ash extract (1:20) and after reaction with ammonium molybdate (Arabi et al., 2014). The concentrations of iron (Fe) and zinc (Zn) were determined with a mass spectrometer (Khan et al., 2017), and copper (Cu) and sulfur (S) were determined by atomic absorption spectrometry (Irschik et al., 2013; Zambrzycka and Godlewska-Żyłkiewicz, 2014).

2.10. Fatty acid profile of ewe meat

Lipids were extracted from 4 g of samples using a methanol:chloroform mixture as described by Bligh and Dyer (1959) modified by Reis et al. (2018). Then 20 mg of lipids was derivatized to obtain fatty acid methyl esters (FAME) according to Hartman and Lago (1973). The FAME were analyzed by injecting 1 μL into a Gas Chromatograph with Flame Ionization Detector (GC-FID) (Varian, Star 3600, USA) and autosampler (Varian, 8200, USA). The carrier gas used was hydrogen at constant pressure of 35 psi. The FAME were separated on an HP-88 capillary column (Agilent Technologies, USA) (100 m × 0.25 mm; 0.20 μm of thickness film) with initial temperature of 50 °C for 1 min, increasing to 185 °C at 15 °C/min and with rate from 0.5 °C/min to 195 °C and finally up to 230–15 °C/min, remaining for 5 min. The detector was kept at 250 °C. Identification was performed by comparing the retention times of compounds with standards FAME Mix 37 (P/N 47885-U), Linoleic acid conjugated methyl ester (P/N O5632), Linoleic acid methyl ester cis/trans isomers (P/N 47791), Linolenic acid methyl ester isomer mix (P/N 47792), Vaccenic acid methyl ester (P/N 46905-U) and Docosapentaenoic methyl ester (P/N 47563-U) (Sigma-Aldrich, USA). Results were expressed as a percentage of the total area taking into account the factors of FID correction and the conversion of esters to acids (Visentainer, 2012).

The sums of saturated FA (∑SFA), polyunsaturated FA (∑PUFA), monounsaturated FA (∑MUFA), FA omega 3 (∑n-3), FA omega 6 (∑n-6), and ratios MUFA:SFA, PUFA:SFA, and n-6:n-3, were estimated. The hypercholesterolemic (H), hypocholesterolemic (h), neutral lipid fatty acids, and residual fatty acids were determined according to Bessa et al. (2015) and the ratio of hypocholesterolemic to hypercholesterolemic (h/H) was determined according to Santos-Silva et al. (2002). The thrombogenic (TI) and atherogenic (AI) index, the activities of the enzymes Δ^9 desaturases C16 and C18 and elongase were calculated (Ulbricht and Southgate, 1991).

2.11. Statistical analysis

The results were analyzed by the PROC GLM of the Statistical Analysis System (SAS University, 2015) analysis of variance and regression at 5% probability, with the decomposition of the sum of squares of the treatments in contrasts related to linear and quadratic effects, with adjustment of the equations of regression. The criteria for choosing the regression models were the significance of the parameters estimated by the models and the values of the determination coefficients (R^2). The standard error of the mean was obtained from the raw data. The following statistical model was used:

$$Y = \mu + Bi + Tj + eij \quad (4)$$

where: μ = overall mean;Bi = effect of block; Tj = effect of different water supply levels; eij = residual error.

3. Results

3.1. Proximate composition, physicochemical characteristics and texture profile of the ewe meat

There was no effect of water restriction on the proximate composition, pH, color parameters (a^*, b^*, W, c^*, and h^*), cooking losses, water holding capacity, and shear force of ewe meat. Water supply reduction resulted in a quadratic effect for L^* in ewe meat ($p < 0.05$), with a maximum value in the water supply of 60%. A quadratic effect was found for resilience ($p < 0.05$). The other characteristics of the meat texture profile were not affected by the treatments (Table 4).

3.2. Mineral composition of ewe meat

Different water supply levels had no influence on N and P in ewe meat. A quadratic effect on K ($p < 0.05$), with lower values in the 60% treatment with 107.50 mg/100 g of meat. Ca showed an increasing linear effect ($p < 0.001$) with the decrease in water supply, corresponding to a 145.2% increase in Ca in the 60% water supply when compared to *ad libitum* water intake (100% treatment). Mg showed a decreasing linear effect ($p < 0.05$), with a reduction of 21.8% in content in the 40% water supply (Table 5).

There was an increase in Na by 0.151 mg/100 g meat for each 1%

Table 4
Physical and chemical characteristics and texture profile of Santa Inês ewe meat receiving different levels of water supply (n = 8).

Variables	Water supply (%)				SEM	*p*-value	
	100	80	60	40		L	Q
Moisture g/100 g	72.5	72.4	72.9	72.5	0.40	0.73	0.57
Ash, g/100 g	1.03	1.04	1.03	1.05	0.02	0.85	0.98
Protein, g/100 g	25.9	24.8	24.1	24.3	0.77	0.12	0.41
Lipids, g/100 g	4.5	4.2	3.7	3.9	0.51	0.29	0.64
pH	5.66	5.60	5.63	5.65	0.03	0.81	0.27
Lightness intensity[a], L*	37.2	40.1	40.4	37.6	1.39	0.79	0.05
Red intensity, a*	12.7	15.0	14.1	14.4	0.77	0.22	0.20
Yellow intensity, b*	10.5	11.5	11.4	10.8	0.55	0.79	0.13
White intensity, W	34.9	37.1	37.7	34.9	1.35	0.90	0.09
Chroma, c*	14.7	17.1	16.2	16.3	0.83	0.29	0.17
Hue angle, h*	16.5	18.9	18.2	18.0	0.91	0.36	0.16
Cooking losses, %	28.5	29.7	31.3	30.5	1.22	0.17	0.41
Water holding capacity, %	36.9	36.9	41.4	38.1	1.13	0.12	0.15
Shear force, kgf/cm^2	10.9	9.9	11.4	10.1	0.48	0.60	0.71
Hardness, N	48.6	31.8	42.2	33.3	7.49	0.07	0.53
Adhesiveness, g/s	-1.9	-2.5	-1.8	-1.8	0.40	0.53	0.53
Springiness, mm	0.9	0.9	0.9	1.0	0.01	0.23	0.36
Cohesiveness* *	0.7	0.7	0.7	0.6	0.01	0.12	0.06
Gumminess, N	32.1	21.2	22.7	18.9	4.58	0.09	0.45
Chewiness, N/mm	28.4	17.3	20.2	21.8	4.94	0.47	0.22
Resilience[b]* *	0.29	0.33	0.30	0.28	0.01	0.20	0.01

SEM=Standard error of the mean; L=Linear; Q=Quadratic. * *dimensionless (Ratios calculated according to Bourne et al., 2002). Significant at 5% probability level. * *dimensionless. Equation: a$\hat{y}$ = 23.566 + 0.496x-0.004x^2, R^2 = 0.99; $\hat{y}$b = 0.1508 + 0.004553x − 0.00030x^2, R^2= 0.98

Table 5
Mineral composition and ratios in meat of Santa Inês ewe receiving different levels of water supply (n = 8).

Mineral (mg/100 g)*	Water supply (%)				SEM	*p*-value		Daily intake for humans[1] (mg/day)	*Source** *
	100	80	60	40		L	Q		
Nitrogen	392.5	403.6	391.4	387.1	1.23	0.27	0.18	–	–
Phosphorus	11.7	11.9	12.9	12.5	0.19	0.41	0.68	500	Franco (2008)
Potassium[a]	174.0	151.5	107.5	113.0	0.87	< 0.001	0.04	3500	Gupta and Gupta (2014)
Calcium[b]	5.7	4.8	13.9	11.9	0.18	< 0.001	0.06	1000	Mahan et al. (2018)
Magnesium[c]	6.4	5.4	5.9	5.0	0.27	0.01	0.76	320–420	Rolim et al. (2020)
Sodium[d]	21.3	29.6	25.7	33.1	0.73	< 0.001	0.42	15,000	Institute of Medicine (2011)
Sulfur[e]	1.9	2.0	2.4	2.3	1.50	0.01	0.38	850	Mahan et al. (2018)
Copper[f]	1.3	0.7	0.6	0.4	0.10	< 0.001	0.07	2–3	Baierle et al. (2010)
Iron[g]	22.8	17.2	11.9	12.1	0.79	< 0.001	< 0.001	10–15	Silva et al. (2018)
Zinc[h]	14.6	13.1	13.7	14.7	0.49	0.67	0.02	11–15	Gupta and Gupta (2014)

SEM=Standard error of the mean; L=Linear; Q=Quadratic. Significant at 5% probability level. *mg/100 g fresh weight; * *Literature data for comparative purposes. Equation: aŷ= 134.050–1.3150x+ 0.018x^2, R^2= 0.91; bŷ= 19.453–0.144x, R^2= 0.54; cŷ= 4.330 + 0.019x, R^2= 0.69; dŷ= 38.427–0.152x. R^2= 0.65; eŷ= 2.759–0.0083x. R^2= 0.75; fŷ= −0.308 + 0.0153x. R^2= 0.89; gŷ= 24.789–0.508x+ 0.005x^2. R^2= 0.98; hŷ= 21.599–0.235x+ 0.00164x^2. R^2= 0.92.

restricted water ($p < 0.001$). The reduction in water supply increased the content of S in the meat ($p < 0.05$), with a maximum point for the water supply of 60% with an increment of 21.3% S in the water supply of 40% compared to *ad libitum* water intake (100% treatment). Cu showed a decreasing linear effect ($p < 0.001$), with a 71.8% reduction in the 40% water supply compared to the *ad libitum* water intake (100% treatment) (Table 5).

A quadratic effect was observed for Fe ($p < 0.001$) and Zn ($p < 0.05$), in which Fe presented a minimum point for the supply of 60%, with 11.95 mg/100 g, corresponding to a reduction of 47.58% in relation to the water supply of *ad libitum* - 100%, while the Zn content reduced by 1.55 mg/100 g in the supply of 80% in relation to the *ad libitum* – 100% treatment (Table 5).

3.3. Fatty acid profile of ewe meat

C14:1 showed a quadratic effect ($p < 0.05$); the ewe receiving 40% water supply had a lower content of C14:1 in meat, in relation to the water supply of 80% and 60%. Water supply levels provided a decreasing linear effect ($p < 0.01$) for C18:1n7t with lower contents in ewe meat that received 40% water supply with a 21.5% reduction when compared to the content of C18:1n7t of the *ad libitum* water intake (100% treatment) (Table 6).

The water supply reduction resulted in a quadratic effect for C20:0 ($p < 0.05$) with higher levels in ewe meat that received 80% water supply with contents of 0.02 g/100 g FA in meat (Table 6). The *ad libitum* water intake (100% treatment) showed a lower C24:0 content with 0.002 g/100 g FA in meat, while higher levels of C24:0 were observed in the 60% water supply treatment (0.018 g/100 g) (Table 6).

Ewe receiving 40% water supply had lower contents of MUFA ($p < 0.05$) in meat, with a reduction of 3.5% in relation to the *ad libitum* water intake (100% treatment). There was no effect of water supply levels on SFA, PUFA, n-3 and n-6. The water supply reduction did not change the TI and AI, in the same way that the activities of Δ^9 desaturase C16, Δ^9

Table 6
Fatty acid composition in meat of Santa Inês ewe receiving different levels of water supply (n = 8).

Fatty acid (FA) (g/100 g FA)	Water supply (%)				SEM	*p*-value	
	100	80	60	40		L	Q
C10:0	0.03	0.03	0.03	0.03	0.002	0.536	0.781
C12:0	0.02	0.02	0.02	0.02	0.001	0.079	0.421
C14:0	1.24	1.34	1.30	1.10	0.090	0.283	0.100
C15:0	0.11	0.12	0.10	0.12	0.010	0.675	0.907
C16:0	25.14	25.14	35.32	24.77	0.504	0.364	0.136
C17:0	0.70	0.82	0.51	0.80	0.080	0.973	0.310
C18:0	20.23	21.67	18.72	23.2	0.970	0.181	0.130
C20:0[a]	0.01	0.02	0.02	0.02	0.002	0.020	0.022
C24:0[b]	0.002	0.02	0.02	0.01	0.002	< 0.001	< 0.001
C14:1[c]	0.02	0.02	0.02	0.01	0.002	0.305	0.027
C16:1	1.48	1.41	1.42	1.20	0.117	0.129	0.523
C17:1	0.38	0.39	0.39	0.36	0.029	0.695	0.479
C18:1 t	0.69	0.80	0.77	0.87	0.088	0.199	0.905
C18:1n9c	47.69	44.46	47.97	44.19	1.040	0.143	0.794
C18:1n7t[d]	0.83	0.74	0.83	0.65	0.037	0.001	0.087
C18:2n6–9t12t	0.04	0.05	0.04	0.05	0.003	0.130	0.215
C18:2n6–9c12t	0.03	0.04	0.03	0.03	0.002	0.430	0.449
C18:2n6–9c12c	1.44	1.72	1.53	1.50	0.176	0.987	0.383
C18:3n6–6c9c12c	0.04	0.05	0.04	0.05	0.003	0.070	0.617
C18:3n3–9c12c15c	0.08	0.09	0.07	0.09	0.013	0.710	0.782
C18:2n6–9c11t	0.04	0.04	0.03	0.03	0.003	0.204	0.997
C18:2n6–8t10c	0.09	0.09	0.08	0.07	0.010	0.307	0.767
C20:3n6	0.03	0.04	0.04	0.03	0.003	0.726	0.165
C22:1n9	0.46	0.59	0.47	0.51	0.068	0.971	0.489
C20:5n3	0.04	0.05	0.05	0.06	0.009	0.127	0.945
C24:1n9	0.04	0.05	0.04	0.04	0.004	0.461	0.274
C22:5n3	0.10	0.13	0.11	0.13	0.016	0.470	0.997
C22:6n3	0.02	0.03	0.03	0.04	0.005	0.102	0.593

SEM=Standard error of the mean; L=Linear; Q=Quadratic; Significant at 5% probability level. Equation: aŷ= −0.0103 + 0.0010x–0.000009x^2, R^2= 0.84; bŷ= −0.021 + 0.0015x–0.000013x^2, R^2= 0.97; cŷ= −0.008 + 0.0008x+ 0.000006x^2, R^2= 0.96; dŷ= 0.604–0.0023x, R^2= 0.50.

desaturase C18 and elongase remained unchanged (Table 7). There was no effect for the ratios UFA: SFA, MUFA: SFA, PUFA: SFA, n-6: n-3 and h/H by the water supply reduction (Table 7).

4. Discussion

Meat color is associated with the quality of the final product and has a great importance by influencing the consumer at the moment of purchase. Despite the effect of reducing the water supply on the lightness in ewe meat, the average values found for this variable fit the pattern described by Esteves et al. (2018), who found an L^* range of 30.03–49.4 in the evaluated Santa Inês ewe sheep meats, and by Pinheiro et al. (2010) who, evaluating the quality of meat from Santa Inês ewes, showed L^* values between 37.56 and 41.13, results that corroborate our findings.

The increase in L^* in the 80% and 60% water supply treatments may have occurred due to the change in the color of the meat being an indication of the net balance between the oxidative, antioxidant and reducing systems in meat (Bekhit et al., 2019). In addition, the muscle surface moisture has the ability to reflect or scatter incident light, promoting changes in the color of the meat (Purslow et al., 2020).

The SF of the ewe meat did not present a significant difference between the treatments, probably due to this parameter being more directly related to the age, breed and sex of the animal. However, the meats were considered hard, since according to Cezar and Sousa (2007), meat fillets that present a cutting pressure above of 3.63 kgf/cm^2 are considered hard. The results found for SF are superior to the studies developed by Esteves et al. (2018) (2.09 kgf/cm^2), Santos et al. (2013) (3.62 kgf/cm^2) and Pinheiro et al. (2010) (4.08 kgf/cm^2) on the quality of Santa Inês ewe meat. The high value for SF may have promoted a lower resilience in the ewe sheep meat studied, so that the water intake of 80% and 60% showed lower resilience of the meat, indicating a decrease in the texture of the ewe sheep meat, resembling a textural class of rubbery meat (Randall et al. 1976).

Table 7
Nutritional classification, ratios of fatty acids and enzymatic activities in *longissimus lumborum* of Santa Inês ewe receiving different levels of water supply (n = 8).

Classification (g/100 g total fatty acids)	Water supply (%)				SEM	*p*-value	
	100	80	60	40		L	Q
SFA	46.48	49.19	46.04	50.08	0.826	0.055	0.435
MUFA[a]	50.96	48.18	51.35	47.47	0.736	0.042	0.466
PUFA	2.60	2.67	2.65	2.48	0.184	0.654	0.530
n-3	0.28	0.30	0.26	0.30	0.026	0.719	0.661
n-6	1.73	1.96	1.72	1.78	0.090	0.786	0.346
H	25.40	26.51	26.65	25.90	0.579	0.534	0.128
h	51.82	49.35	52.38	48.71	0.775	0.089	0.450
Neutral	21.75	23.13	20.19	24.44	0.844	0.195	0.109
Residual	1.68	1.78	1.49	1.63	0.106	0.371	0.845
TI	1.73	1.91	1.70	1.98	0.059	0.067	0.410
AI	0.30	0.31	0.31	0.30	0.008	0.919	0.072
Δ^9 desaturase C16	5.76	5.31	5.28	4.58	0.393	0.058	0.764
Δ^9 desaturase C18	70.14	67.25	71.90	65.65	1.211	0.123	0.185
Elongase Ratio	72.61	71.36	71.38	72.17	0.571	0.611	0.093
MUFA:SFA	1.11	0.98	1.12	0.95	0.034	0.054	0.556
PUFA:SFA	0.06	0.05	0.06	0.05	0.005	0.595	0.634
n-6:*n*-3	7.25	7.07	7.04	6.10	0.504	0.141	0.462
h/H	2.06	1.87	1.97	1.89	0.055	0.112	0.381

SFA=Saturated fatty acids; MUFA=Monounsaturated fatty acids; PUFA=Polyunsaturated fatty acids; n-3 =Omega 3; n-6 =Omega 6; h=hypocholesterolemic; H=hypercholesterolemic; TI=thrombogenic index; AI=atherogenic index; SEM=Standard error of the mean; L=Linear; Q=Quadratic; Significant at 5% probability level. Equation: $\hat{ay}$= 46.941–0.0364x, R^2= 0.23.

The high levels of K in meat in the 100% and 80% treatments can be indicative of maintaining a positive cation balance, through the regulation of input (food and water) and output (feces and urine) electrolytes (More and Sahni, 1978). K^+ can be lost through breathing in order to maintain osmotic pressure and balance between the body's fluids (Chedid et al., 2014), a factor that explains the reduction in K for treatments of 60% and 40% water supply (Table 6). Kasap et al. (2018), when analyzing the mineral composition of the meat of female ewes, found a K concentration of 310.82 mg/100 g of meat, a result superior to those recorded in this study. The daily requirement of K for humans is 3500 mg for the body to present a balance in electrolyte metabolism (Gupta and Gupta, 2014). Thus, with the reduction of water supply for ewe's (from 60% to 40% of water supply) there was a reduction of this mineral.

The increase in Ca levels with the decrease in water supply may be related to muscle activity post-mortem, in the movement of fibers and direct action with calpain that are dependent on Ca in its activity of protein renewal and meat tenderization during aging (Bhat et al., 2018). Phosphorylation, on the other hand, also compromises the regulation of μ-calpain, however the increase in Ca^{2+} concentration decreases the effects of phosphorylation (Du et al., 2017), a factor that may have inhibited the increase in the contents of phosphorus in meat of the animals studied. Another factor that may have contributed to the increase in Ca retention may have been through the mobilization of Ca from bone tissue and transported *via* the vascular system for metabolic use during the stress process, in order to keep the neural and enzymatic activity intact (Du et al., 2017). The recommended Ca intake for adult humans is 1000 mg daily (Mahan et al., 2018). This is the amount of Ca needed to carry out the metabolic function of transport and muscle contraction. In this way, the production of ewe meat under different water supplies is a viable alternative for consumption, since ewe meat presents an increase of 108.7% with the supply of 40% of water, when compared to the *ad libitum* water supply (100%).

The Mg can influence the tenderness of meat due to its interaction with Ca and calpains, by reducing the action of stress, with the increase in Mg, promoting the reduction of blood concentrations of cortisol and catecholamine (D'Souza et al., 1998) and making the decrease of water in the organism not enough to change the physical characteristics of the meat, since the Ca:Mg ratio remained high in the 80%, 60% and 40% water supply levels when compared to the 100% treatment. Magnesium is a highly necessary nutrient in human nutrition, requiring between 320 and 420 mg of magnesium daily to maintain enzyme functions, cell development and muscle movement (Rolim et al., 2020). In this context, sheep meat is essential in the formation of the human diet to meet the requirements of these nutrients.

The increase in Na in meat may have occurred due to its role in osmolarity of the system, which allows for greater retention of this mineral in the body, and at each rehydration period, it promotes an increase in the urinary concentration/retention of Na (Rakova et al., 2017), thus contributing to the accumulation of this mineral in muscle tissues. According to the Institute of Medicine (2011), the recommended total sodium intake is 15,000 mg/day for an adult. The amount of Na found in sheep meat is much lower than recommended for daily intake, but the NaCl commonly used for the preparation of this food can meet the nutritional requirements without compromising human health.

The increase in S may be associated with catabolism of proteins into amino acids, even though in the present study there was no effect of water supply levels on protein content. Muscle proteolysis is important for the metabolism of adaptation and increase of muscle mass, promoting a balance between protein synthesis and breakdown rates (Tipton et al., 2018). The breakdown of muscle protein leads to the production of water and the release of amino acids through peptidases, which promotes the release of amino acids with S in their composition. In this sense, protein catabolism will make amino acids available for synthesis of vital tissues and for regeneration of muscle tissues (Cobos and Diaz, 2015). The S plays an important role in the metabolism of

carbohydrates, fats, and vitamins. This mineral is of great importance in the human body. adult humans require 850 mg/day of this mineral (Mahan et al., 2018).

Copper is essential for maintaining the lipid quality of meat, due to its pro-oxidant action on lipid components, which can act on the direct oxidative activity of fatty acids (Schuhmann-Irschik et al., 2015). As the water volume for animal watering was reduced, Cu density per unit of water ingested increased, an effect that may have compromised the passive absorption by the body and decreased the mineral accumulation in the muscle tissue. The human body retains most copper in the brain, liver, kidneys, blood and skeletal muscle tissue. The daily intake for the maintenance of these organs and tissues in humans is 2–3 mg/day according to the World Health Organization (WHO) (Baierle et al., 2010), demonstrating that ewe meat with or without water restriction is a good source of copper.

The decrease in Fe content can cause changes in the oxidative balance of meat in addition to changes in color (Buzała et al., 2016), a fact not observed in the present study, since the intensity of redness was similar between the treatments studied. The contents of Fe in this study were higher than those reported by Pannier et al. (2014) for sheep with 2.13 mg/100 g, and for lambs with 2.03 mg/100 g. Ewe meat is considered a good source of iron in human nutrition. In humans, iron is essential for hematological functions, with the daily requirement of doses between 40 and 50 mg/day of iron, and in a balanced diet the absorption is 10–15 mg/day (Silva et al., 2018). Therefore, when we offer only 40% of the supply water to the ewe, we reduce the Fe content in their meat, therefore, it is necessary to increase the consumption of ewe meat to meet the daily need.

Zinc plays a key role in muscle growth, keratin production and collagen synthesis (Mehdi and Dufrasne, 2016). The Zn content showed a recovery rate of 100.88% when the water supply was reduced to 40%, demonstrating that this level of water supply enabled zinc content higher than in the *ad libitum* supply. The Zn contents are higher than those of Pannier et al. (2014), with values from 1.18 to 4.49 mg/100 meat, for animals from different crosses with the Merino breed. The human body needs 11–15 mg/day of Zn in this sense, all treatments in this study are able to provide the amount of Zn needed by our body (Gupta and Gupta, 2014).

The most prevalent SFA observed in this study were C14:0, C16:0 and C18:0, these SFA are predominant in sheep meat (Cruz et al., 2011). C14:0 is classified as hypercholesterolemic; C16:0 is less hypercholesterolemic and responsible for the increase in cholesterol; C18:0, in the metabolism, can be converted into C18:1 that regulates serum levels of HDL cholesterol (Oliveira et al., 2018). C20:0, together with its derivatives, acts as a precursor to lipoxins that promote the reduction of inflammation in the body, in addition to having immune function and acts in the development of the retina and brain during pregnancy and childhood in humans (Seah et al., 2017). The values obtained for C20:0 were lower than those observed by Vargas Junior et al. (2019), who found 0.07 g/100 FA of C20:0 in crossbred Santa Inês and Pantaneiro sheep.

C24:0 plays a fundamental role in the homeostatic activity of the liver (Raichur et al., 2014), an effect that may have promoted its accumulation in the muscle of animals subjected to water restriction, in order to maintain homeorhesis amidst hydro-electrochemical changes in the body. Studies developed by Yousfi et al. (2016) evaluated the effect of salts in drinking water for lambs and reported contents of 0.71% of C24:0 in meat, being higher than those found in this study.

The 40% reduction in water supply resulted in a lower MUFA content. This is similar to the findings of Santos et al. (2019), in Santa Inês lambs (47.46%). The accumulation of PUFA and MUFA in ruminant meat is intrinsic to ruminal activity and metabolism, driven by microbial action and lipid biohydrogenation. Thus, the absorption of PUFA in the digestive tract of ruminants is limited by the activity of biohydrogenation while the activity of biohydrogenation together with the enzymatic activity of the rumen microbiological population favors the deposition of MUFA in muscle tissue (Turner et al., 2015).

Considered one of the most abundant acids among UFAs, C18:1n9c has a beneficial effect on human health by decreasing LDL cholesterol and reducing cardiovascular diseases (Wang et al., 2018). On water restriction for Santa Inês lambs, Santos et al. (2019) also observed no changes in the C18:1n9c content when subjected the animals to intervals of up to 72-h of restriction, presenting an average of 40.25%, being lower than that observed in this study.

The reduction in C18:1n7t may be associated with less ruminal biohydrogenation activity, through the biohydrogenation of C18:2n-6 and C18:3n-3 in the rumen (Hiller, 2014), indicating that the decrease in the supply and renewal of fluids in the rumen alters the dynamics of FA production. Such an effect may be related to the osmolarity of the ruminal fluid and cation absorption rate. Vaccenic acid is a precursor of CLA, which is produced by the muscle tissue of ruminants (Smith et al., 2009), a factor that may also have attributed to the lower content of trans-vaccenic fatty acid and CLA in this study when compared to Santos et al. (2019) with 0.15% CLA (C18:2c9t11) for animals under constant cycles of water restriction for 72-h.

The h/H ratio showed an average value of 1.94, considered low, according to the classification of Santos-Silva et al. (2002), for whom the reference value for the h/H ratio is 2.0. The TI and AI were not influenced by water supply, however the values were low for both AI and TI, close to those reported by Borghi et al. (2016) with 0.82 for atherogenic index, and 1.88 for thrombogenic index, in Ile de France lambs.

5. Conclusion

Water supply levels of up to 40% voluntary intake can be recommended for short periods, up to 60 days, in the event of low water availability, as a management strategy in the animal confinement. Independent of the level of water supply, the texture profile and proximate composition meet the standards of quality meat for sheep.

The content of Ca, Na and S increased with reduced water supply, and the availability of fatty acids was maintained. The water supply of up to 60% voluntary intake provides greater lightness and MUFA and a lower concentration of K and Fe in ewe meat. The water supply in up to 80% of the voluntary intake provides a lower concentration of Zn in ewe meat. There was no change in fatty acids with hypercholesterolemic potential. However, the MUFA content was reduced in the 40% treatment.

Finally, this research makes possible a way out for periods of water scarcity, conditioning the animals to a water management that is inferior to their physiological needs and that allows the commercialization of animals for human consumption with a quality product, instead of having the loss of the animal from severe dehydration.

CRediT authorship contribution statement

Cleyton de Almeida Araújo: Formal analysis, Methodology, Investigation, Writing – original draft. **Gherman Garcia Leal de Araújo:** Project administration, Conceptualization, Supervision, Visualization, Resources, **André Luiz Rodrigues Magalhães:** Conceptualization, Supervision, Resources. **Fleming Sena Campos:** Supervision, Visualization, Writing – review & editing. **Glayciane Costa Gois:** Writing – original draft, Writing – review & editing. **Maria Helena Tavares de Matos:** Project administration, Conceptualization, Supervision, Resources. **Deneson Oliveira Lima:** Formal analysis, Methodology, Investigation. **Rafael Torres de Souza Rodrigues:** Writing – review & editing. **Cedenir Pereira de Quadros:** Conceptualization, Supervision, Resources. **Roger Wagner:** Conceptualization, Supervision, Resources. **Raquel Guidetti Vendruscolo:** Methodology, Investigation.

Conflict of interest

The authors declare that they have no competing interests.

Data Availability

The data that support this study will be shared upon reasonable request to the corresponding author.

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