Correlation between mineral profile, physical-chemical characteristics, and proximate composition of meat from Santa Ines ewes under water restriction

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Correlation between mineral profile, physical-chemical characteristics, and proximate composition of meat from Santa Ines ewes under water restriction

Correlação entre perfil mineral, características físico-químicas e composição centesimal da carne de ovelhas Santa Inês submetidas à restrição hídrica

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Highlights

Water restriction changed the correlation between N and other minerals in ewe meat.
Gumminess and chewiness had a positive correlation with N in ewe meat.
Mg showed a negative correlation with lipid content in the 60% water supply.

Abstract

This study aimed to evaluate the correlations between mineral profile, physical and chemical characteristics, and proximate composition of ewe meat receiving different water supply levels (100% - *ad libitum* group; 80%; 60% and 40% *ad libitum* group). Thirty-two Santa Ines ewes were assigned to a randomized block design, with 4 treatments, and 8 replications, during the 63-day experimental period. Significant correlations

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between all minerals (P<0.05) were found in the 60% and 40% water supply levels. A correlation (P<0.05) was observed for minerals P, K, Ca, Mg, S, Cu, and Fe with crude protein at 100% water supply. Negative correlations (P<0.05) between N, P, K, Ca, Mg, S, Cu, Fe, and Zn were detected in the meat of animals supplied with 60% water. Principal component analysis (PCA) of macrominerals explained 82.9% data variance. Zinc had a strong contribution to PC1. Cooking losses had a similar contribution to PC1 and PC2. PC1 and PC2 explained 66.7% data variance in chemical characteristics. The decrease in water supply causes the correlation of nitrogen with the other minerals in meat, in addition to altering the correlation between the physical and chemical profile of the meat.

Key words: Calcium. Crude protein. Hardness. Iron. Principal componentes.

Resumo

O objetivo deste estudo foi avaliar as correlações entre o perfil mineral, as características físico-químicas e a composição centesimal da carne de ovelhas recebendo diferentes níveis de oferta hídrica (100% - grupo *ad libitum*; 80%; 60% e 40% do grupo *ad libitum*). Trinta e duas ovelhas Santa Inês foram distribuídas em delineamento em blocos ao acaso, com 4 tratamentos e 8 repetições, durante o período experimental de 63 dias. Correlações significativas entre todos os minerais (P<0,05) foram encontradas nos níveis de 60% e 40% de oferta hídrica. Observou-se correlação (P<0,05) dos minerais P, K, Ca, Mg, S, Cu e Fe com a proteína bruta em 100% de oferta hídrica. Correlações negativas (P<0,05) entre N, P, K, Ca, Mg, S, Cu, Fe e Zn foram detectadas na carne de animais que receberam oferta de 60% de água. A análise de componentes principais (ACP) dos macrominerais explicaram 82,9% da variação dos dados. O zinco apresentou forte contribuição para a componente 1 (CP1). As perdas por cozimento tiveram contribuição semelhante para CP1 e componente 2 (CP2). A CP1 e a CP2 explicaram 66,7% da variação dos dados das características químicas. A diminuição da oferta hídrica promoveu a correlação do nitrogênio com os demais minerais da carne, além de alterar a correlação entre o perfil físico e químico da carne.

Palavras-chave: Cálcio. Componentes principais. Dureza. Ferro. Proteína bruta.

Introduction

Climate change alters the hydrological cycle (Tercini et al., 2021) resulting in an imbalanced distribution of fresh water in dryland regions (Wu et al., 2021). Consequently, in many countries of the world, especially in arid and semi-arid areas (Middle East, North Africa, South Asia, and Northern South America), water is a scarce resource, which affects efficiency and threatens sustainability in production systems (Ibidhi & Ben Salem, 2018). Agriculture is the largest user (over 70% total water) of freshwater resources, and global livestock production represents about 30% global water needs (Ran et al., 2016).

Sheep meat production plays an important role in human nutrition, as a source of protein of high biological value. Feedlot systems have become more frequent, due to the possibility of developing nutritional management, ensuring meat production throughout the year (Ribeiro et al., 2020). However, water scarcity is a limiting factor for sheep production in feedlot systems in dryland regions. Ibidhi and Ben Salem (2018) highlighted that water use for sheep meat production is 6.98 and 9.01 m^3 kg^{-1} body weight in production systems located in wet and semi-arid regions, respectively. Moreover, water use for animal production can become increasingly restricted in qualitative and quantitative terms (Chedid et al., 2014). Thus, studies on the minimum water levels required by ruminants in feedlot systems, especially in semi-arid regions, make it possible to know the relationship between the water demand necessary for sheep to express their potential to transform muscle into meat, without affecting health.

In sheep, water restriction for 72 hours causes changes in meat tenderness, requiring a shear force of 15.29 N cm^{-2} for cutting, but does not alter the chemical composition of the meat (Santos et al., 2019). Estimates of correlations for physical-chemical and proximate characteristics of meat are essential for knowing the dynamics of antagonism and synergism between biomolecules constituting the nutritional profile of sheep meat. Some studies bring the correlations between untargeted metabolomics and cooking loss, chewability, protein, and fat content (X. Zhang et al., 2022), and correlations between genetic traits and meat quality (Mortimer et al., 2018). However, correlations between minerals in ewe meat, especially from animals that have undergone water restriction, are still scarce.

Thus, this study hypothesized that the reduction in water supply alters the correlations between the chemical and physical components of meat. Therefore, the aim was to evaluate the correlations between the mineral profile, physical and chemical characteristics, and proximate composition of the meat of sheep receiving different water supply levels.

Material and Methods

Experiment location and ethical aspects

The experiment was conducted at the Universidade Federal do Vale do São Francisco - UNIVASF. All procedures followed the protocols approved by the Animal Research Ethics Committee of UNIVASF (0002/241017). The present research is part of a larger project with a methodology based on Araújo et al. (2022).

Animals, treatments and experimental diets

Thirty-two Santa Ines ewes, multiparous, non-lactating, with 2.3 ± 1.0 years of age, average body weight of 32.2 ± 7.4 kg, were subjected to four water treatments: water ad libitum (100%), 80% offer; 60% offer and 40% offer of the ad libitum group. Animals were housed in folds and adapted to diets and management for 14 days. Subsequently, the trial lasted 63 days. A randomized block design with 4 treatments and 8 replications was adopted. The initial body weight was used to define the blocks.

The experimental diet consisted of fresh Cameroon elephant grass (*Pennisetum purpureum* Schum) and concentrate. The elephant grass came from an experimental area and was manually cut 10 cm above the ground. The material was cut daily and processed in a stationary forage machine (Nogueira Pecus 9004, Saltinho - SP, Brazil) to obtain 2.5 cm particles. The diet was formulated with a forage: concentrate ratio of 46:54 on a dry matter basis (Table 1), to obtain gains of 157g day^{-1}, to meet the nutritional requirements of multiparous, non-lactating, dual-purpose ewes, following the recommendations of the National Research Council [NRC] (2007). The diet was offered daily (09h00 and 15h00), and the water at 09h00. The amount of food supplied was calculated based on the intake of the previous day, not allowing leftovers greater than 15% of the quantity offered.

Table 1
Chemical composition of ingredients and diet and experimental diet composition

Items (in g kg^{-1} dry matter)	Elephant grass	Ground corn	Soybean meal	Diet
Dry matter*	261.9	889.3	886.1	576.3
Ash	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	15.5	370.6
Acid detergent fiber	419.5	33.7	88.5	206.9
Total carbohydrates	830.5	859.9	42.8	715.3
Non-fiber carbohydrates	174.0	642.0	27.8	328.3
Total digestible nutrients	570.1	850.0	80.5	596.7
Experimental diet		in g kg^{-1} dry matter		
Elephant grass		460.0		
Ground corn		381.0		
Soybean meal		132.0		
Urea		7.0		
Mineral supplement[1]		20.0		

[1] Guarantee levels per kilo of product guaranteed by the manufacturer: calcium (min.) 190g; phosphorus (min.) 75g; magnesium (min.) 10g; chlorine (min.) 218g; sulfur (min.) 70g; sodium (min.) 143g; copper (min.) 300mg; cobalt (min.) 405mg; iron (min.) 500mg; iodine (min.) 80mg; manganese (min.) 1,100mg; selenium (min.) 30mg; zinc (min.) 4600mg; fluorine (max.) 0.87g; solubility of phosphorus (P) in 2% citric acid (min.): 95%. * in g kg-1 natural matter.

Slaughter and loin collection

At the end of the trial, animals were fasted (solid and liquid) for 16 hours, weighed, and stunned by brain concussion. After bleeding, skinning, and evisceration (Carvalho et al., 2015), carcasses were cooled (± 4 °C, 24h00) and the loins were separated, packed in plastic bags, and kept under refrigeration (- 20 °C). The loins had an average weight of 0.657 g. The *Longissimus lumborum* muscle was crosswise cut along its length between the 12th and 13th ribs. Samples of *L. lumborum* muscle from each animal were dried and ground (MARCONI mill, model MA 350/70/NYL) to determine the content of nitrogen - N (Association of Official Analytical Chemists [AOAC], 2016) (Method 981.10); sodium - Na and calcium - Ca (American Oil Chemists Society [AOCS], 1995) (Method

15b-87); potassium - K and magnesium - Mg (Harris, 1991); phosphorus - P (Arabi et al., 2014); iron - Fe, and zinc - Zn (Khan et al., 2017), copper - Cu (Irschik et al., 2013), and sulfur - S (Zambrzycka & Godlewska-Żyłkiewicz, 2014).

Analyses were performed using fresh samples. Approximately 50 grams of loin was used for the analysis of chemical composition, anticipating possible repetitions. After sample processing in a processor (Ph900v turbo, 250w, Red, 220V, Philco), moisture (MOI, Method 985.41) was quantified by weighing 2.0 grams of meat and drying it in an oven with air circulation at 105 °C for 16 hours. Protein content (CP, Method 928.08) was determined in 0.5 grams of meat by digestion with sulfuric acid and then titration with hydrochloric acid. The lipid content (LIP) was determined in 1.0 grams of meat according to AOCS (2017) through extraction with petroleum ether. All analyses were performed in triplicate.

The pH was determined according to AOAC (2016) using a portable digital pH meter (TESTO-205, Testo SE & Co. KGaA, Campinas, São Paulo, Brazil), previously calibrated with two standards (pH 4.0 and 7.0), at the center of the samples. Meat color was analyzed in 2.5 cm thick loin samples, thawed under refrigeration (8 °C), and previously exposed to oxygen for 30 minutes at room temperature before reading. After 30 minutes, color values were measured at three points on the surface of the sample using a MINOLTA CR-400 colorimeter (Konica® Minolta, Osaka, Japan), and lightness (*L**), redness (*a**) and yellowness (*b**) were determined (Van Laack et al., 2000). The colorimeter was calibrated using a white calibration with the D65 illuminant at 10° for standard observation, being operated in an open cone.

Cooking loss (CL) was determined using meat samples of approximately 1.0 cm^3, cut parallel to muscle fibers (three technical replicates), following the methodology of Piñero et al. (2008). Samples presented initial weights between 2.44 grams and 2.84 grams. They were cooked in a digital water bath (TECNAL, Piracicaba, SP, Brazil) until the internal sample temperature reached 72 ºC, measured using a copper-constant thermocouple equipped with a digital reader. Values of cooking loss were obtained by the difference between the fresh sample mass and the cooked sample mass. The water holding capacity (WHC) was determined according to Honikel and Hamm (1994), using 0.5 g of previously cooked muscle, subsequently pressed with a weight of 5 kg for 5 minutes with the aid of acrylic plates; after this pressing, samples were weighed again.

The texture profile was determined using a TA-XT Plus texturometer (Stable Micro System Ltd, Vienna Court, United Kingdom), according to Huidobro et al. (2005). Samples of 20 mm in diameter and 20 mm in thickness were placed in a water bath until reaching an internal temperature of 72 °C. After cooking, the sample was taken to the equipment for determination of adhesiveness (ADE), springiness (SPR), hardness (HARD), cohesiveness (COH), chewiness (CHE), gumminess (GUM) and resilience (RES), using the Texture Expert® software (Bourne, 2002).

Statistical analysis

Pearson correlation coefficients (r) were estimated for all meat traits assuming intervals of -1 (negative linear association) and 1 (positive linear association). Data were analyzed by t-test using PROC GLM and PROC CORR from the Statistical Analysis System Software [SAS] (2015) at 5% and 1% probability levels. To generate indices and group individuals according to their variances, multivariate analysis was performed for exploratory purposes. In Principal Component Analysis (PCA), treatments were plotted against the first two components (PC1 and PC2) using the software RStudio (2020) version 3.3.1, using the FactoMiner and Factoextra packages. To construct the graphs, the SigmaPlot software version 2014 was used.

Results and Discussion

There was no correlation ($P>0.05$) between N content and mineral contents in the Santa Ines sheep meat in the treatment of 100% water supply. There was also no correlation between P content and K, Mg, S, Zn, and Na contents, and between Na and Cu contents in the sheep meat from animals receiving 100% water supply. In contrast, there was a positive association between P content and the minerals evaluated (Table 2).

With the reduction in the water supply to 80%, there was no correlation ($P>0.05$) between N and Ca and between N and S, however, there was a positive association ($P<0.05$) between all other minerals. For N, a moderate correlation ($P<0.05$) was detected with P ($r = 0.79$); Cu ($r = 0.73$), and Zn ($r = 0.78$) contents, while there was a strong correlation ($P<0.001$) with K ($r = 0.93$) and Mg ($r = 0.84$), Fe ($r = 0.86$) and Na ($r = 0.95$). For the treatment with 60% water supply, only Na and Cu contents were not correlated ($P>0.05$). On the other hand, the content of N was strongly correlated ($P<0.001$) with seven minerals (P, K, Ca, Mg, S, Fe, and Zn) and moderately correlated with Cu ($r=0.79$) and Na ($r=0.78$). When the water supply was 40%, there was a positive association between all the minerals evaluated, highlighting a strong and positive correlation for the contents of P, K, Mg, S, Fe, Zn, and Na (Table 2).

According to Baaij et al. (2015), P has a positive correlation with K and Mg. This may be related to their abundance in the animal organism, where K and Mg are the most abundant cations in the intracellular environment, participating in several metabolic reactions, such as enzymatic reactions, especially in phosphorylation. Potassium is the main intracellular cation active in the metabolism and synthesis of glycogen and proteins, also acting in the regulation of the body's water content, showing a strong relationship with Na and water due to its hygroscopic and fluid regulation capacity. Thus, the filtrate in the glomerulus corresponds to about 65% filtered load, conferring reabsorption in the proximal convoluted tubule due to the association of water and Na reabsorption (Hoskote et al., 2008). This relationship between K and Na is favorable in meat, considering that Na is found in scarce amounts.

Table 2
Pearson's correlation coefficients between the mineral profile of the meat of Santa Ines ewes subjected to water supply restriction

	N	P	K	Ca	Mg	S	Cu	Fe	Zn
Water ad libitum (100%)									
P	-0.10								
K	0.30	0.64							
Ca	0.12	0.92***	0.86**						
Mg	0.29	0.62	0.94***	0.81**					
S	0.29	0.66	0.99***	0.88**	0.93***				
Cu	0.47	0.79*	0.84**	0.92***	0.78**	0.86**			
Fe	0.15	0.87**	0.90***	0.95***	0.85**	0.91***	0.88**		
Zn	0.28	0.58	0.94***	0.78**	0.91**	0.96***	0.77*	0.86**	
Na	0.16	0.58	0.89**	0.75**	0.79*	0.90**	0.70	0.85**	0.95***
80% offer									
P	0.79*								
K	0.93***	0.92***							
Ca	0.61	0.91***	0.79*						
Mg	0.84**	0.95***	0.93***	0.91***					
S	0.66	0.92***	0.82*	0.99***	0.92***				
Cu	0.73*	0.94***	0.88**	0.97***	0.97***	0.97***			
Fe	0.86***	0.93***	0.94***	0.90***	0.94***	0.92***	0.93***		
Zn	0.78*	0.93**	0.89***	0.90***	0.89***	0.90***	0.89**	0.96***	
Na	0.95***	0.89**	0.95***	0.75*	0.94***	0.77*	0.85**	0.90***	0.84***
60% offer									
P	0.96***								
K	0.94***	0.98***							
Ca	0.96***	0.96***	0.93***						
Mg	0.93***	0.95***	0.91***	0.92***					
S	0.93***	0.97***	0.97***	0.90**	0.95***				
Cu	0.79*	0.86**	0.89***	0.72*	0.78*	0.92***			
Fe	0.85**	0.92***	0.94***	0.80*	0.85**	0.96***	0.97***		
Zn	0.92***	0.97***	0.96***	0.89**	0.94***	0.99***	0.92***	0.95***	
Na	0.78*	0.79*	0.76*	0.83*	0.85**	0.78*	0.59	0.70*	0.72*
40% offer									
P	0.89**								
K	0.83**	0.95***							
Ca	0.78*	0.90***	0.91***						
Mg	0.82*	0.95***	0.94***	0.97***					

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S	0.90***	0.95***	0.96***	0.89**	0.94***				
Cu	0.69*	0.84**	0.91**	0.92**	0.90**	0.82*			
Fe	0.90***	0.98***	0.92***	0.92***	0.96***	0.93***	0.81*		
Zn	0.83**	0.91***	0.91***	0.99***	0.97***	0.90**	0.90**	0.94***	
Na	0.93***	0.98***	0.90**	0.83***	0.90***	0.93***	0.76*	0.97***	0.85**

N= nitrogen; P=phosphorus; K=potassium; Ca=calcium; Mg=magnesium; S=sulphur; Cu=copper; Fe= iron; Zn=zinc Na=sodium. ***Significant (P<0.001) ** (P<0.01) *(P<0.05) by the "t" test.

Calcium dynamics in animal cells vary depending on the site. Intracellular Ca is present in the cytoplasm, in the extracellular medium and inside the cell in the nuclear matrix, and in the mitochondrial matrix, so the Ca^{2+} content at lower levels is regulated by ATPase that transports Ca^{2+} from the membrane, in addition to the action of the Na+: Ca^{2+} exchanger in resting cells (Bagur et al., 2018). However, Ca^{2+} regulation can also be impaired by Zn dynamics, where Zn overload can disrupt Ca^{2+} homeostasis (Guo et al., 2014). This effect may demonstrate the search for homeorhesis of the cell membrane of skeletal tissue, given that there was an increase in the correlation between Ca and Zn as the water supply decreased. In this context, Ca plays a key role in muscle activity and the transformation of muscle into meat, activation of calpains that act in meat tenderization, however a subset of Ca transporters and signalers must be activated (Jiang et al., 2019).

The correlation between Ca and Mg is associated with ATP activity and substrate availability, so that Mg^{2+} assumes the role of substrate in a complex that requires the presence of ATP for cardiac Ca^{2+}-ATPases, thus altering the affinity of some exchangers, such as Na^{+}-Ca^{2+} type 1 for Ca^{2+} (Baaij et al., 2015). The opening of the permeability transition pore of the mitochondrial membrane is usually performed by the generated reactive oxygen species and with Ca^{2+} overload, which triggers a series of apoptotic biochemical reactions altering mitochondrial functions and muscle softening in the *post-mortem* (Wang et al., 2017).

The correlation between Ca and Fe is related to the action of the mitochondrial Ca uniporter that causes homeostasis between the two minerals (L. Zhang et al., 2019a). Some studies associated the accumulation of Fe in the cell with the oxidative process and neurological alterations due to mitochondrial dysfunction (Liang et al., 2008). In addition, Fe reduction in leaner animals is related to a reduction in muscle aerobic activity and myoglobin levels (Knight et al., 2020). This effect can change meat color, due to the chemical characteristics of iron and the binder at the last site, determining the color of the meat (Lawrie, 2017). Thus, the increase in Ca retention occurs through the mobilization of bone tissue and is transported via the vascular system for metabolic use during the stress process, in order to keep neural and enzymatic activity intact (Du et al., 2017).

Similar to Duan et al. (2015) on beef cattle, correlations were observed between Zn and Mg at all levels of water supply tested. Among the cations present in the cell, Mg is considered the most abundant, after K. In this sense, its correlation with the other minerals evaluated in this study is associated with biomolecule anabolism and catabolism, in addition to modulating the selectivity of membrane channels, conferring the permeability to a monovalent cation, such as Na (Romani, 2011). Like Ca, Mg acts *in rigor* mortis, in the pre-rigor where ATPs are complexed with Mg^{2+}, later the phase of physical alterations begins, giving the muscle an inelastic and inextensible capacity (Lawrie, 2017).

Among the processes of interaction between minerals, the dynamics of S ingestion and absorption can interfere with the rate of absorption and accumulation in the cell. Underwood and Suttle (1990) stated that, in ruminants, the absorption of Cu is influenced by the S and Fe content in the diet. Copper helps in iron absorption, which is necessary for energy production, antioxidant, and cholesterol regulator, being influenced by Zn, so that Zn has a direct action on metallothionein that regulates the Zn and Cu interaction, in addition to acting as a site of storage and detoxification (Hill & Shannon, 2019). Our results corroborate Duan et al. (2015), who observed positive correlations between Zn and Fe, and Mg in the beef *Longissimus* muscle. According to Gussarsson and Jensen (1992), copper has the ability to inhibit the influx of K^+, whereas the passive influx of K^+ also decreases with the absorption of Cu.

In the evaluation of the physical-chemical composition of the meat, no correlation ($P>0.05$) was found between the contents of N, Mg, and Zn and the contents of LIP, MOI, CP, and the values of pH, L^*, a^* and b^* in the 100% water supply (Table 3). However, there was a correlation ($P<0.05$) between the CP content of the meat with six minerals (P, K, Ca, S, Cu, and Fe) and a negative correlation ($P<0.05$) between meat pH and Na content. For the treatment with 80% supply, there was a correlation between the CP content and the minerals P, K, Mg, Cu, Fe, Zn, and Na (Table 3). The water supply at 60% resulted in a negative correlation ($P<0.05$) between lipid content and N, P, K, Ca, Mg, S, Fe, and Zn (Table 3). In contrast, there was a moderate and positive correlation ($P<0.05$) between meat moisture and P and K contents; between meat CP and nine minerals (P, K, Ca, Mg, S, Cu, Fe, Zn, and Na) and between pH and P, K, S, Cu, Fe and Zn contents. Meat color was not correlated ($P>0.05$) with the mineral content in the meat of sheep under different water supply levels (Table 3). For the treatment with 40% water supply, there was a positive correlation ($P<0.05$) between pH and N, K, and S contents (Table 3).

Table 3
Pearson's correlation coefficients between mineral profile and physicochemical composition of meat of Santa Ines ewes subjected to water supply restriction

	N	P	K	Ca	Mg	S	Cu	Fe	Zn	Na
Water ad libitum (100%)										
LIP	0.08	-0.55	-0.57	-0.62	-0.61	-0.63	-0.52	-0.53	-0.67	-0.59
MOI	-0.06	0.29	0.55	0.42	0.55	0.59	0.35	0.45	0.68	0.64
CP	1.00	0.99***	0.70*	0.94***	0.67	0.73*	0.84**	0.91*	0.66	0.66
pH	0.02	-0.05	-0.47	-0.24	-0.29	-0.50	-0.19	-0.30	-0.60	-0.73*
L*	0.04	0.00	-0.02	0.12	-0.11	-0.07	0.14	-0.1	-0.06	-0.03
a*	-0.14	-0.54	-0.27	-0.40	-0.19	-0.22	-0.43	-0.55	-0.18	-0.26
b*	-0.03	-0.19	-0.09	-0.05	-0.09	-0.03	-0.06	-0.30	-0.11	-0.17
80% offer										
LIP	-0.09	0.03	-0.01	-0.13	-0.22	-0.08	-0.19	-0.08	-0.03	-0.19
MOI	-0.05	-0.12	0.08	0.07	0.10	0.00	0.09	0.01	0.02	0.04
CP	1.00	0.79*	0.93***	0.61	0.84**	0.66	0.73*	0.86**	0.78*	0.95**
pH	0.16	0.36	0.30	0.38	0.45	0.31	0.44	0.30	0.33	0.31
L*	-0.11	0.08	-0.01	-0.02	0.02	-0.04	0.00	-0.08	-0.09	-0.08
a*	0.00	0.46	0.20	0.38	0.28	0.34	0.33	0.22	0.29	0.12
b*	0.00	0.40	0.13	0.35	0.22	0.33	0.29	0.16	0.22	0.04
60% offer										
LIP	-0.86**	-0.88**	-0.88**	-0.88**	-0.79*	-0.81*	-0.68	-0.80**	-0.82*	-0.68
MOI	0.66	0.71*	0.75*	0.66	0.53	0.67	0.69	0.77*	0.66	0.55
CP	1.00	0.96***	0.94***	0.96***	0.93***	0.93***	0.79*	0.85**	0.92***	0.78*
pH	0.66	0.77*	0.79*	0.63	0.64	0.82**	0.94***	0.90***	0.82*	0.49
L*	0.04	0.01	-0.01	-0.08	0.07	0.09	0.13	0.20	0.10	0.08
a*	-0.04	-0.14	-0.22	-0.08	-0.15	-0.22	-0.32	-0.24	-0.16	-0.26
b*	0.18	0.03	-0.01	0.06	0.06	0.00	-0.12	0.00	-0.03	-0.02
40% offer										
LIP	-0.24	-0.10	-0.12	-0.11	-0.04	-0.01	-0.27	-0.08	-0.13	-0.12
MOI	-0.12	-0.09	-0.16	-0.01	-0.13	-0.21	-0.06	-0.05	-0.01	-0.13
CP	1.00	0.13	0.08	0.31	0.20	0.14	0.27	0.20	0.36	0.16
pH	0.70*	0.67	0.74*	0.50	0.61	0.82*	0.53	0.59	0.51	0.68
L*	0.29	0.23	0.22	0.45	0.38	0.23	0.29	0.34	0.47	0.22
a*	0.13	0.31	0.38	0.57	0.41	0.22	0.53	0.37	0.54	0.19
b*	0.06	0.16	0.27	0.48	0.32	0.11	0.50	0.23	0.46	0.06

N= nitrogen; P= phosphorus; K= potassium; Ca= calcium; Mg= magnesium; S= sulphur; Cu= copper; Fe= iron; Zn= zinc Na= sodium; LIP= lipids; MOI= humidity; CP= crude protein; pH= Hydrogenonic potential; L*= Brightness intensity; a*= Red intensity; b*= Yellow intensity. Significant at the level *** (P<0.001) ** (P<0.01) *(P<0.05) by the "t" test.

Meat CP content interacted synergistically with the contents of P, K, Ca, S, Cu, Fe, Mg, and Na in the water supply of 100, 80 and 60%, that is, as the meat CP content decreases, there may also be a reduction in the concentration of these minerals. The Ca/CP ratio is associated with the high functionality performed by proteins, so that the protein can bind Ca^{2+}, conferring the binding of the Ca^{2+} group to helix-loop-helix domains with different functionalities (Gifford et al., 2007). In addition, proteins bind to Ca^{2+} and assume the function of Ca^{2+} buffer proteins that determine the synchrony and action of Ca^{2+} signals, imparting Ca^{2+} homeostasis (Bagur et al., 2018).

The CP content is related to non-heme iron, which is found in ferritin and CP with iron in their composition (Duan et al., 2015), so it is possible to state that protein in the water supply of 100 and 60% had more bonds with the iron group, which can change the color of the meat, making it darker (Lawrie, 2017).

Another positive correlation related to CP content was the association with Zn. This effect highlights the importance of the porphyrin ring and heme iron, which act as essential factors in the absorption of Zn and Fe, promoting their availability (Stipanuk & Caudill, 2018). Magnesium showed a negative correlation with lipid content in the 60% water supply treatment, which is similar to the findings of Duan et al. (2015) in beef cattle. The authors attributed this effect to the action of Mg as a critical cofactor in several enzymes associated with energy metabolism, glucose utilization, and ATPase function.

No correlations (P>0.05) were found between minerals and the meat texture properties in the 100% supply (Table 4). The meat COH of animals that received 80% water supply showed a negative correlation (P<0.05) with seven minerals (P, K, Ca, Mg, S, Cu, and Fe). However, with the reduction to 60 and 40% levels, no correlations (P>0.05) were detected between COH and meat minerals (Table 4). Meat GUM of sheep receiving 80% water supply showed negative correlations (P<0.05) with N, P, Mg, and Na. Chewiness also showed a negative correlation (P<0.05) with N (r =-0.74) and Na (r=-0.81), while meat resilience had a negative correlation (P<0.05) with Ca (r = -0.69) and S (r =-0.66) (Table 4). The reduction in the concentration of these minerals in meat results in greater cohesiveness, gumminess, chewiness, and resilience, which results in tougher meat.

For the 60% water supply, there was a negative correlation (P<0.05) between WHC and N, P, K, M, and Zn, an effect not observed at the 40% supply level (Table 4). The antagonistic relationship between WHC and the contents of N, P, K, Mg, and Zn can be related to changes in pH. According to Jacob and Pethick (2014), pH is the main factor influencing WHC, with minimum retention capacity at the isoelectric point of meat proteins (pH 5.0-5.5). In turn, the decrease in N and K concentrations may be related to a drop-in meat pH, which causes denaturation and loss of solubility of muscle proteins, consequently, these proteins lose their ability to attract water (Y. Zhang & Ertbjerg, 2019b; X. Zhang et al., 2022).

Table 4
Pearson's correlation coefficients between mineral profile and physical and textural characteristics of meat from Santa Ines ewes subjected to water supply restriction

	N	P	K	Ca	Mg	S	Cu	Fe	Zn	Na
Water *ad libitum* (100%)										
CL	0.56	-0.04	0.05	0.06	0.19	0.07	0.22	-0.04	0.04	-0.10
WHC	0.55	-0.62	-0.47	-0.53	-0.53	-0.47	-0.23	-0.60	-0.52	-0.55
HARD	-0.21	0.52	0.02	0.27	0.12	0.05	0.19	0.33	0.13	0.12
ADE	-0.52	-0.03	-0.06	-0.15	-0.11	-0.05	-0.33	0.01	0.16	0.33
SPR	-0.30	-0.15	-0.16	-0.23	-0.15	-0.10	-0.32	-0.13	0.13	0.25
COH	-0.29	0.33	-0.02	0.17	0.00	0.06	0.06	0.15	0.23	0.32
GUM	-0.22	0.56	0.07	0.32	-0.20	0.11	0.23	0.37	0.21	0.19
CHE	-0.24	0.48	0.04	0.25	0.15	0.08	0.15	0.31	0.21	0.21
RES	-0.03	0.26	0.04	0.21	0.09	0.14	0.17	0.10	0.24	0.26
80% offer										
CL	-0.60	-0.32	-0.42	-0.20	-0.47	-0.19	-0.33	-0.36	-0.34	-0.64
WHC	-0.09	0.13	0.03	0.15	-0.07	0.16	0.01	0.16	0.25	-0.18
HARD	-0.52	-0.45	-0.40	-0.34	-0.50	-0.32	-0.41	0.30	-0.40	-0.63
ADE	0.08	0.33	0.33	0.29	0.20	0.29	0.27	-0.36	0.33	0.07
SPR	-0.53	-0.46	-0.42	-0.32	-0.49	-0.31	-0.40	-0.36	-0.37	-0.64
COH	-0.54	-0.86**	-0.75*	-0.80**	-0.79*	-0.81*	-0.83**	-0.70*	-0.68	-0.69
GUM	-0.79**	-0.64*	-0.65	-0.56	-0.73*	-0.59	-0.64	-0.68	-0.62	-0.82**
CHE	-0.74*	-0.60	-0.61	-0.49	-0.68	-0.50	-0.54	-0.60	-0.56	-0.81*
RES	-0.04	-0.60	-0.31	-0.69*	0.49	-0.66*	-0.61	-0.38	-0.42	-0.26
60% offer										
CL	0.64	0.44	0.40	0.57	0.41	0.36	0.20	0.26	0.34	0.38
WHC	-0.71*	-0.70*	-0.69*	-0.67	-0.75*	-0.68	-0.48	-0.56	-0.70*	-0.48
HARD	-0.03	-0.02	0.04	-0.09	-0.23	0.06	0.09	0.01	0.01	-0.58
ADE	-0.54	-0.59	-0.62	-0.54	-0.52	-0.54	-0.43	-0.52	-0.58	-0.25
SPR	0.21	0.35	0.27	0.44	0.38	0.25	0.06	0.14	0.24	0.53
COH	-0.27	-0.37	-0.45	-0.28	-0.13	-0.31	-0.41	-0.41	-0.36	0.12
GUM	-0.06	-0.05	0.01	-0.12	-0.27	-0.10	0.04	-0.03	-0.03	-0.61
CHE	-0.04	-0.01	0.04	-0.07	-0.24	-0.08	0.05	-0.02	-0.05	-0.58
RES	-0.34	-0.39	-0.47	-0.30	-0.11	-0.34	-0.50	-0.48	-0.37	0.05
40% offer										
CL	-0.61	-0.72*	-0.71*	-0.70*	-0.66	-0.60	-0.76*	-0.70*	-0.70*	-0.68
WHC	0.13	0.18	0.36	0.12	0.17	0.27	0.45	0.06	0.09	0.15
HARD	0.44	0.22	0.25	-0.05	0.04	0.36	-0.04	0.14	-0.01	0.32
ADE	0.49	0.35	0.33	0.54	0.47	0.36	0.37	0.39	0.51	0.23
SPR	0.41	0.48	0.37	0.61	0.61	0.51	0.41	0.55	0.61	0.45

continue...

continuation...

COH	0.58	0.47	0.32	0.50	0.49	0.31	0.49	0.51	0.50	0.46
GUM	0.89**	0.44	0.51	0.21	0.28	0.53	0.26	0.37	0.25	0.51
CHE	0.34	0.84**	0.83**	0.86**	0.88**	0.92***	0.71*	0.87**	0.89**	0.84**
RES	-0.31	0.38	0.23	0.50	0.48	0.29	-0.31	0.45	0.50	0.36

N= nitrogen; P= phosphorus; K= potassium; Ca= calcium; Mg= magnesium; S= sulphur; Cu= copper; Fe= iron; Zn= zinc Na= sodium; CL = cooking losses; WHC= water holding capacity; HARD= hardness; ADE= adhesiveness; ELA= elasticity; COH= cohesiveness; GUM= gummy; CHE= chewability; RES= Resilience.
Significant at the level *** ($P<0.001$) ** ($P<0.01$) *($P<0.05$) by the "t" test.

Meat texture parameters are highly related to available Ca levels, because in the tenderization process, during the breakdown of muscle fibers, Ca is released to activate proteases (calpains), which require high concentrations of Ca to be activated, thus accelerating the meat tenderization process (Knight et al., 2014). For 40% water supply, there was a negative correlation ($P<0.05$) between CL and P, K, Ca, Cu, Fe, and Zn contents, and this is due to physical, chemical, and structural changes in meat components resulting from the heat (Rosa et al., 2006). Factors, such as temperature, cooking method, and duration of the process are responsible for these changes in the chemical composition and consequently in the nutritional value of the meat (García-Arias et al., 2003).

Nevertheless, gumminess and chewiness showed a synergistic correlation with N and GUM (r = 0.89), and between CHE and P (r=0.84), K (r=0.83), Ca (r=0.86), Mg (r=0.86), S (r=0.92), Cu (r=0.71), Fe (r=0.87), Zn (r=0.89) and Na (r=0.84) (Table 4). This result may be related to a possible increase in WHC, which leads to tougher and darker meat, with higher values of gumminess and chewiness, requiring the application of greater force to make the meat ready to be swallowed.

The PCA analysis demonstrates the distribution of vertices on a multivariable space, so that close variables have high correlations, variables distributed in the same quadrant are positively correlated, and when arranged on the opposite side of the origin, are negatively correlated. The PCA analysis of microminerals summarized 85.5% total variance in the first two principal components (30.1% and 55.4%, respectively for PC1 and PC2, Figure 1A). Thus, the distribution of the variables of water supply levels in the first two PCs was different in the multivariate space, so that Na and S presented a greater contribution in the 40% supply treatment (Figure 1A), mainly contributing to PC2. Iron and Cu presented higher values in the 100% supply treatment, with a strong correlation with each other. Likewise, Fe and Cu had equivalent contributions to PC1 and PC2 (Figure 1A). Zinc, in turn, presented a strong contribution to PC1, when projected perpendicular to the Zn vector, higher contents were found for the 40% supply compared to 60; 80, and 100% supply treatments (Figure 1A).

The PCA analysis of macrominerals (Figure 1B) summarized 82.9% variance, with 61.9% represented by PC1, and 21.0% by PC2. In this sense, N had a greater contribution to the formation of PC2, while Mg had a greater contribution to PC1 (Figure 1B). Calcium and P, in turn, presented greater values in the 60% supply treatment, positively contributing to PC1. The physical profile of the meat showed a variability of 38.0% for PC1 and 26.1% for PC2 (Figure 2A).

Meat CHE and HARD corresponded to the principal components of the sheep meat receiving water ad libitum (100% supply). In turn, GUM was higher in the meat of sheep subjected to the 60% water supply treatment (Figure 2A). Cooking losses had a similar contribution to PC1 and PC2, the increase in cooking losses can be achieved in water supplies of 80 and 60%.

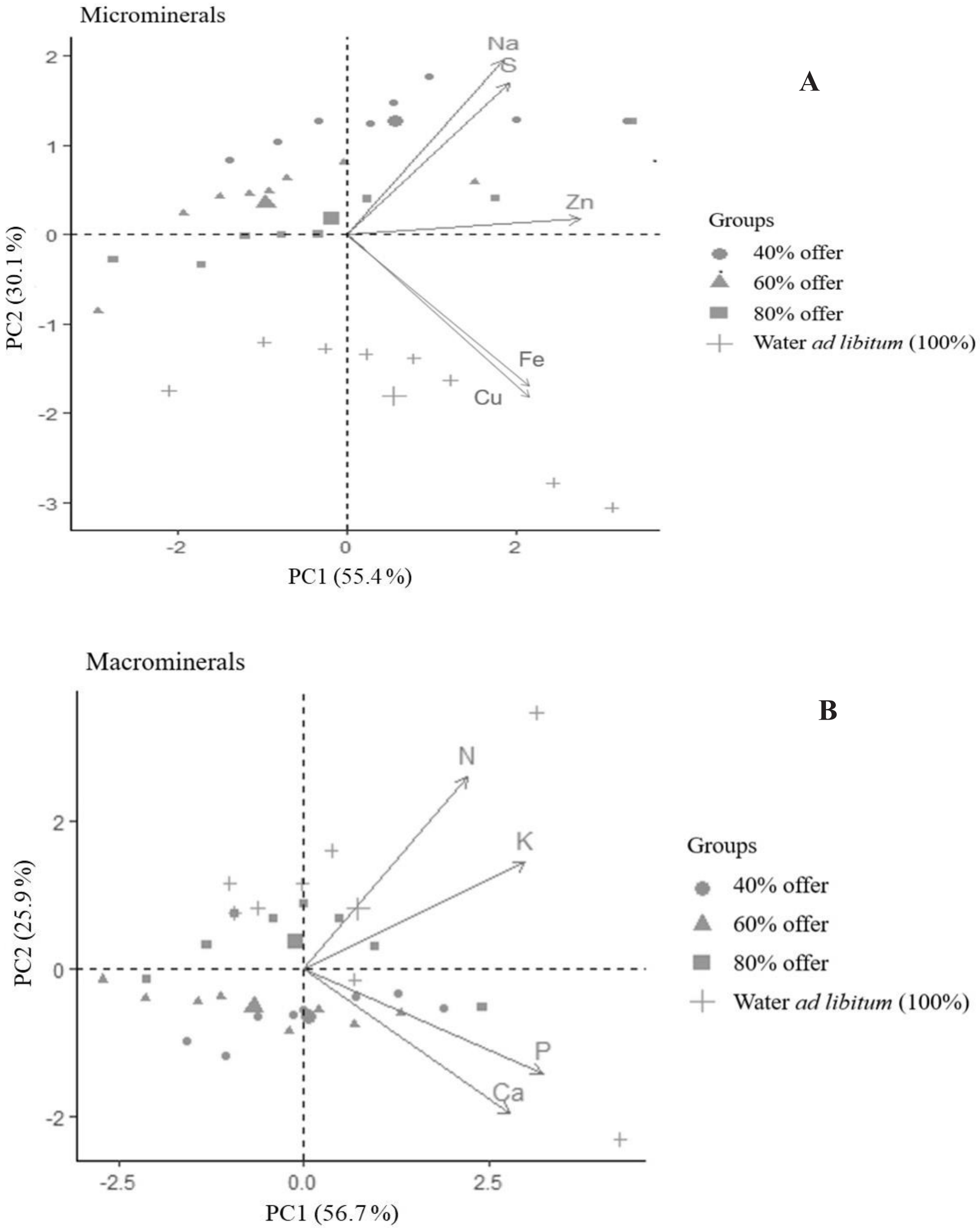

Figure 1. Principal Components and percentage of variance explained by the components (PC1 and PC2) of the Micro (A) and Macro (B) minerals of meat from Santa Ines ewes subjected to water supply restriction.

In the evaluation of chemical properties, the first two principal components (PC1 and PC2) explained 66.7% data variance. The results of this analysis allowed to infer that the highest LIP content was achieved in the 100% water supply treatment, being antagonistic to the moisture content (Figure 2B). It is possible to achieve higher levels of *L**, *a** and *b** when using 40% water supply strategies, also increasing meat pH (Figure 2B).

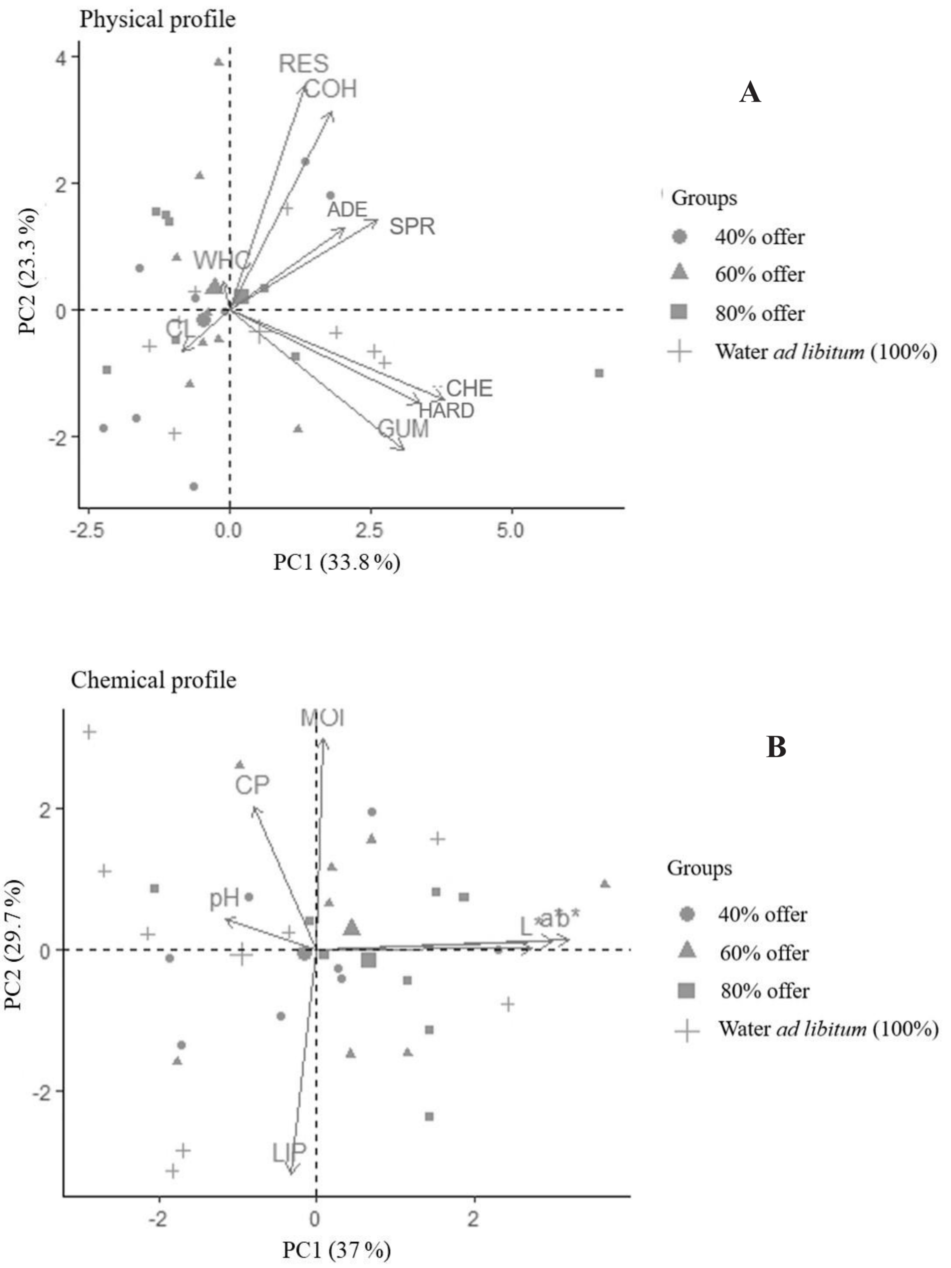

Figure 2. Principal Components and percentage of variance explained by the components (PC1 and PC2) of the Physical (A) and Chemical (B) minerals of meat from Santa Ines ewes subjected to water supply restriction.

Conclusions

The reduction in water supply alters the correlation between nitrogen and other minerals, promoting greater synergism when the supply is reduced to 40% voluntary intake. Correlation analysis indicates that there is an increase in the correlation between the chemical composition of food and the reduction in water supply, with a strong relationship between minerals and the chewiness of meat from animals with low water intake.

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