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Mineral profiling of muscle and hepatic tissues of Australian Merino, Damara and Dorper lambs: Effect of weight loss

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Abstract

Seasonal weight loss (SWL) is a major constraint to extensive animal production systems. The Australian sheep production is based on merino sheep, a European breed not tolerant to SWL. Tolerant alternative breeds such as the fat-tailed Damara and the Dorper have been increasingly used in Australia and elsewhere, due to their robustness. The aim of this study was to understand the mineral profile of muscle and liver tissues of Australian Merino, Damara and Dorper, when subjected to SWL in order to understand SWL-tolerance physiology. Twenty-four lambs were divided randomly between growing (control) and nutritionally restricted groups for each breed. The trial lasted 42 days. Animals were weighed bi-weekly and at the end of the trial, lambs were slaughtered. Liver and muscle samples were taken immediately after slaughter. Mineral assessment was carried out using inductively coupled plasma–optical emission spectrometry. Analysis of variance showed mineral concentrations were generally increased in the muscle of restricted animals, mainly because of fat tissue mobilization. An increase in Zn and Fe concentrations indicates an increase of enzymatic activity in the liver of restricted sheep as well as differential abundance of Fe-containing proteins. High concentrations of Cu in the liver of Dorper indicate higher ability to accumulate this element, even under SWL.

1 INTRODUCTION

In tropical and Mediterranean countries, ruminant production systems are largely based in biomass production available in natural pastures. As these systems are subjected to the influences of various natural factors, pasture availability and quality can fluctuate significantly throughout the year. In the dry season, digestibility of these pastures decreases significantly, triggering seasonal weight loss (SWL) that can arise up to 30% of the animals' initial live weight. It has an important impact on animal production systems in these areas, as supplementary feed is often necessary if not indispensable to prevent loss of production (Almeida et al., **2013**). This phenomenon has been studied in goat bucks (Almeida, Schwalbach, Waal, Greyling, & Cardoso, **2006**), sheep (Almeida et al., **2013**, **2016**; Ferreira et al., **2017**; Miller et al., **2019**; Ribeiro, Madeira, et al., **2019**; Scanlon et al., **2013**) and dairy goats (Hernández-Castellano et al., **2016**; Lérias et al., **2013**, **2015**; Palma, Hernández-Castellano, et al., **2016**). Specifically, Scanlon et al. (**2013**) have reported sheep performance of Australian Merino, Damara and Dorper lambs. They showed that the Australian Merino, a wool-producing and SWL-susceptible breed, lost most weight when under SWL, while Damara and Dorper lost equal percentages of initial live weight (LW). Dorper lambs outperformed the other two breeds among growth groups, while the Damara had the worst performance. The Damara, a fat-tailed breed, appears to be the most adapted to SWL among these three. The Dorper, a South African composite breed of Persian Black Head and Dorset Horn, has an intermediate adaptation (Almeida, **2011**).

Nutrition represents a significant aspect in the concentration of macro and micro minerals of edible tissues of numerous species (Ribeiro, Mourato, & Almeida, **2019**). It also affects the capability of tissues to properly carry out the metabolic processes along the metabolic pathways as these minerals are used structurally and as co-factors of enzymes by tissue cells (Kincaid, **1999**). The fat tail of Damara lambs under SWL has been shown to increase Ca and Zn concentrations in comparison to growing animals (Lérias et al., **2016**). Boer goat buck carcasses, when fed with supplements to veld (*Themeda trianda*) hay, had less Mg (175.81 mg/100 g), Fe (12.09 mg/100 g), Ca (573 mg/100 g) and P (3,345.5 mg/100 g) compared with restricted animals with 225.73, 15.96, 787 and 4,227.4 mg/100 g of carcass for each mineral respectively (Almeida et al., **2006**). A similar relationship has been found in steers finished on either feedlot or pasture systems (Freitas et al., **2014**), where Zn concentration was higher in pasture-finished steers. These differences among nutritional groups resulted from endogenous nutrient mobilization and lessened accumulation of adipose tissue in nutritionally restricted animals and in pasture-fed animals. The presence of fat, a tissue with low mineral contents by comparison with either muscle or hepatic tissue, dilutes these elements in animal tissues. This has been shown by Williams et al. (**1983**) for the soft tissue of forage and grain-finished steers, the latter having lower concentrations of Zn, P, Mg and K.

The objective of this study is to evaluate the influence of SWL in the mineral profiles of muscle and liver tissues of Australian Merino, Damara and Dorper lambs and to extrapolate physiological implications in mineral metabolism of these tissues, for evaluation of SWL-tolerance mechanisms of relevance for the industry.

2 MATERIALS AND METHODS

2.1 Live animal experiment

The experimental design was described by Scanlon et al. (**2013**). Briefly, the animal trial was carried out at the Merredin Dryland Research Institute in Western Australia (Merredin, WA, Australia; Latitude: 31°28′51″S; Longitude: 118°16′28″E and elevation above sea level: 320 m) during the spring (September–October 2007). Seventy-two lambs of three breeds (Australian Merino, Damara and Dorper) were used. Twenty-four animals per breed were randomly split into two nutritional levels: growing and restricted groups of 12 animals each. Nutritional treatments were calculated using the Freer equation so that growing and restricted lambs would gain or lose (85% maintenance needs) 100 g of live weight/day respectively. Australian and European Union procedures on animal experimentation were followed (Process 07ME06). Animals were weighed twice each week during the trial period of 42 days: to adjust feed ration to live weight and to measure animal performance. They were fed individually on the same diet of a pelleted compound feed (Macco 101, Macco Feeds, Williams, WA, Australia). At the end of the trial, the animals were slaughtered, and liver and *Gastrocnemius* muscle samples were taken and snap-frozen in liquid nitrogen. They were kept at −80°C until further analysis.

2.2 Sample preparation and digestion

Approximately 1 g of both tissues was weighed and dried until constant weight at 65°C. Once dried, all samples were ground with a mortar and pestle until having a fine homogeneous powder and weighed in a digestion tube (50 ml) to an approximate weight of 0.3 g.

Sample dissolution was performed as an adaptation of the method described by Roselli, Desideri, Meli, Fagiolino, and Feduzi (**2016**). Briefly, 3 ml of concentrated nitric acid, 10 ml of hydrochloric acid and 1 ml of hydrogen peroxide were added to each digestion tube. Samples were left in the acids for 16 hr, and hydrogen peroxide was added immediately before digestion, to avoid sample loss due to the reaction between the latter and the acids.

After the acid mixture was added, tubes were distributed randomly in a digestion plate (DigiPREP MS, SCP Science) in which they were heated following the pattern: 1 hr to reach 95°C and then 1 hr at 95°C. After a total digestion time of 2 hr, samples were allowed to cool off in a ventilated chamber.

Once at room temperature, samples were diluted with distilled water in a volumetric flask (25 ml). Diluted samples were filtered into sealed flasks using filter papers with a diameter of 90 mm (Filter-Lab ref. 1242, FILTROS ANOIA S.A.).

2.3 ICP-OES readings

Inductively coupled plasma–optical emission spectrometry (ICP-OES) readings were performed in an iCAP 7000 Series, ICP-OES spectrometer (Thermo Scientific), equipped with an automated sampler. Multi-element standards (SPC Science, PlasmaQual S22) were used to create the calibration curves necessary to quantify the different elements.

Multi-element detection and quantification took place overnight, to detect the following elements: Sn (tin), V (vanadium), Li (lithium), Ba (barium), Se (selenium), As (arsenic), Co (cobalt), Zn (zinc), Fe (iron), Mn (manganese), Cu (copper), Pb (lead), Cd (cadmium), Ni (nickel), Cr (chromium), S (sulphur), P (phosphorous), Mg (magnesium), Ca (calcium), K (potassium) and Na (sodium). No further dilution was needed for any element before analysis. Other elements, Sn, V, Li, Ba, As, Pb, Cd, Ni and Cr, are presented separately.

2.4 Statistical analysis

Two-factor variance analysis was carried out using the general linear model procedure of SAS system, 3rd edition (SAS Institute Inc.): breed, nutritional level and the interaction between the two factors were fitted. When significant *p* values ($p < .05$) of the interaction were obtained, mineral concentrations were compared (separately) using the least square means procedure. The Univariate procedure was used to obtain standard error of the means (*SEM*). Atypical values were not considered in some mineral element readings for both tissues.

3 RESULTS AND DISCUSSION

Mineral profiling has been extensively studied before, in the most varied contexts and species (Almeida et al., **2006**; Giuffrida-Mendoza, Arenas de Moreno, Uzcátegui-Bracho, Rincón-Villalobos, & Huerta-Leidenz, **2007**; Lérias et al., **2016**; Mahmud, Rehman, Anwar, & Ali, **2011**). This particular study has been performed at the end of a series of previously published papers (Almeida et al., **2013**; Almeida et al., **2016**; Ferreira et al., **2017**; Miller et al., **2019**; Palma, Scanlon, et al., **2016**;

Ribeiro, Madeira, et al., **2019**; Scanlon et al., **2013**) which allows the interaction of our results to others concerning SWL and tissue proteomics, metabolomics, amino acid quantification, animal performance and meat quality.

3.1 Muscle mineral status

Muscle mineral status is presented in Tables **1** and **2** for macro and micro mineral elements, respectively, and in Table **3** for the other elements. Some mineral elements differ significantly between feeding levels of the same breed, albeit no significant interaction was found between breed and nutrition for most of these minerals with the exception of Ba. Restricted lambs had higher concentrations of Ca, Fe, Na and Zn. For example, this difference was found between feeding levels in the Dorper: 188.77 and 135.23 mg Ca/kg DM for restricted and growing lambs respectively. In Australian Merinos, restricted lambs had 157.96 mg Zn/kg DM, significantly more than the 120 mg Zn/kg DM obtained for growing lambs. Similar trends have been reported in Boer goat buck carcasses (Almeida et al., **2006**) and the fat tail of Damara lambs (Lérias et al., **2016**). Williams et al. (**1983**) found that grain-finished steers had 15% more fat in their soft tissues, by comparison, to forage finished steers. This resulted in lower concentrations of Zn, P, Mg and K in the tissues of fatter steers. During SWL, lambs are expected to lose weight as a result of muscle protein breakdown and fatty tissue mobilization towards the liver, for energy production (Van Harten et al., **2013**). Indeed, Almeida et al. (**2013**) have reported that all breed groups of this trial, when restricted, have lost more than 10% of their initial LW. Hence, most differences are likely explained by adipose tissue (IMF) mobilization. Indeed, restricted Australian Merino, Damara and Dorper lambs have started with 32.9, 42 and 37.9 kg of initial LW and by day 42 of this trial they weighed 28.6, 37.3 and 34.3 kg of final LW respectively.

TABLE 1. Macromineral concentration (mg/kg dry matter) in the *Gastrocnemius* muscle of lambs from three different sheep breeds (Australian Merino, Damara and Dorper): growth and restricted

Mineral	Australian Merino		Damara		Dorper		*SEM*	*p* Value (Breed)	*p* V (fe lev
	Restricted	Growth	Restricted	Growth	Restricted	Growth			
Calcium (Ca)	200.53	188.71	187.76	166.77	188.77	135.23	4.89	*	**

Mineral	Australian Merino		Damara		Dorper		*SEM*	*p* Value (Breed)	*p* V (fe lev
	Restricted	Growth	Restricted	Growth	Restricted	Growth			
Potassium (K)	14,727.34	14,593.39	13,802.74	12,755.76	12,935.72	12,366.49	309.08	*	NS
Magnesium (Mg)	909.47	916.93	897.82	872.08	864.46	725.70	26.26	NS	NS
Sodium (Na)	2,421.27	2,284.91	2,814.66	2,172.80	2,437.03	1,983.91	51.94	*	**
Phosphorous (P)	7,171.83	7,029.14	6,849.68	6,429.05	6,929.16	5,426.99	190.53	NS	NS
Sulphur (S)	7,295.92	7,107.61	8,149.05	8,184.93	7,243.31	5,712.74	223.49	**	NS

- Abbreviation: NS, non-significant.
- * $p < .05$; ** $p < .01$.

TABLE 2. Micromineral concentration (mg/kg dry matter) in the *Gastrocnemius* muscle of lambs from three different sheep breeds (Australian Merino, Damara and Dorper): growth and restricted

Mineral	Australian Merino		Damara		Dorper		*SEM*	*p* Value (breed)	*p* Value (feedin level)
	Restricted	Growth	Restricted	Growth	Restricted	Growth			
Cobalt (Co)	0.28	0.22	0.34	0.27	0.24	0.17	0.01	*	*
Copper (Cu)	5.48	5.64	6.68	5.46	4.43	3.83	0.20	**	NS

Mineral	Australian Merino		Damara		Dorper		*SEM*	*p* Value (breed)	*p* Value (feeding level)
	Restricted	Growth	Restricted	Growth	Restricted	Growth			
Iron (Fe)	107.61	71.55	109.79	105.05	77.60	65.15	3.82	**	**
Manganese (Mn)	0.59	0.45	0.66	0.69	0.44	0.40	0.03	**	NS
Selenium (Se)	0.46	0.44	0.63	0.46	0.49	0.31	0.03	NS	NS
Zinc (Zn)	157.96	120.00	132.89	141.25	117.18	90.05	5.10	**	*

- Abbreviation: NS, non-significant.
- * $p < .05$; ** $p < .01$.

TABLE 3. Other element concentrations (mg/kg dry matter) in the *Gastrocnemius* muscle of lambs from three different sheep breeds (Australian Merino, Damara and Dorper): growth and restricted

Mineral	Australian Merino		Damara		Dorper		*SEM*	*p* Value (breed)	*p* Value (feeding level)
	Restricted	Growth	Restricted	Growth	Restricted	Growth			
Arsenic (As)	2.02	1.84	1.91	2.04	1.70	1.68	0.07	NS	NS
Barium (Ba)	2.01^{a}	1.77^{ac}	2.84^{b}	1.54^{ac}	1.80^{ac}	1.40^{c}	0.10	*	**
Cadmium (Cd)	0.05	0.05	0.05	0.04	0.04	0.04	0.0025	NS	NS

Mineral	Australian Merino		Damara		Dorper		*SEM*	*p* Value (breed)	*p* Valu (feedin level)
	Restricted	Growth	Restricted	Growth	Restricted	Growth			
Chromium (Cr)	2.36	2.17	2.42	2.11	1.89	1.66	0.08	*	NS
Lithium (Li)	0.93	0.75	1.14	0.73	0.34	0.46	0.07	**	NS
Nickel (Ni)	2.55	2.11	2.59	2.04	2.51	2.09	0.15	NS	NS
Lead (Pb)	0.47	0.55	0.57	0.58	0.41	0.49	0.03	NS	NS
Tin (Sn)	2.24	2.80	4.43	2.62	4.13	1.77	0.26	NS	*
Vanadium (V)	0.44	0.39	0.50	0.46	0.35	0.32	0.02	*	NS

Note

- Values with different superscripts indicate significant differences ($p < .05$) when the interaction is significant.
- Abbreviation: NS, non-significant.
- * $p < .05$; ** $p < .01$.

Potassium was the most abundant mineral in this tissue, with concentrations higher than 10,000 mg K/kg DM muscle, regardless of breed or nutritional level. This was also observed for other mammalian species such as dromedaries (El-Faer, Rawdah, Attar, & Dawson, **1991**), horses (Grace, Pearce, Firth, & Fennessy, **1999**), cattle and buffalo (Giuffrida-Mendoza et al., **2007**), pigs and wild boar (Sales & Kotrba, **2013**), donkey (Polidori, Vincenzetti, Cavallucci, & Beghelli, **2008**) and avian species such as the ostrich (Sales & Hayes, **1996**). The obtained results can be explained because this element is an intracellular ion crucial to allow muscle stimuli (Suttle, **2010**) and the level

of underfeeding was possibly not high enough to compromise this function. The insufficient level of nutritional restriction could also be the cause for the lack of significant interactions between the two factors tested.

Another noticeable difference is the fact that Damara sheep's muscle is more concentrated in Fe than groups of other breeds, with the exception of restricted Australian Merinos. In fact, both groups of the Damara breed had over 100 mg Fe/kg DM. Restricted Australian Merinos had similar values, most likely resulting from IMF mobilization, as explained previously. Iron is a mineral that is part of myoglobin that is related to oxidative fibres and oxidative metabolism in the muscle. In addition, Ferreira et al. (**2017**) have reported a higher abundance of ferritin heavy chain in the muscle of restricted (vs. growing) Damaras and Dorper that were involved in this study. This is an iron-carrying protein, which partially explains the differences verified for muscle Fe concentrations. Indeed, Pannier et al. (**2014**) stated that selection of lambs to produce leaner meat increases the proportion of glycolytic fibres in the muscle, thus reducing the number of oxidative ones. The Damara breed has suffered no selection towards higher growth rates and fat accumulation, which is why its meat appears to be similar to game and dark-coloured, as reported by Almeida et al. (**2013**). Its meat is darker because myoglobin is relatively more abundant in comparison to Dorper and Australian Merino. Moreover, Damara is more susceptible to stress, which could contribute to darker colour because of low meat pH and the formation of lactic acid pre-slaughter. Haemoglobin is also present in greater concentration in Damara muscles, because this breed originates from the Kalahari Desert, where they must endure long distances in the search of feed (Almeida, **2011**). Growing Damaras had higher concentrations of Zn (141.25 mg Zn/kg DM vs. 90.05 mg Zn/kg DM) than growing Dorper lambs. Pannier et al. (**2014**) showed that both Fe and Zn increase with an increase in isocitrate dehydrogenase activity (Pannier et al., **2014**), a mitochondrial enzyme involved in the formation of NADH and $FADH_2$ when ATP demand of cells increases (Denton, **2009**). The evaluation of this enzyme's activity would bring further insight to the results obtained for Fe and Zn.

Calcium concentration in growing Damara lambs (166.77 mg Ca/kg DM) was higher than in growing Dorper lambs (135.23 mg Ca/kg DM). Increased Ca concentration is characteristic of energy-dependent cellular functions, such as muscle contraction and cell proliferation (Denton, **2009**). Dietary Ca requirements of lambs, albeit decreasing with age, increase with growth rate (Suttle, **2010**). The difference between Ca concentration in the muscle of the mentioned breeds might result from this rather being a breed effect, since Dorper lambs have increased growth rates in comparison to Damara ones (Almeida, **2011**; Scanlon et al., **2013**), thus prioritizing Ca for skeleton growth.

Significant differences exist between breeds for some of the other element concentrations in the muscle (Table 3). The differences indicate that these breeds accumulate Ba and Li (for example) differently. Similar trends have been reported previously in the concentration of Cd in goat tissues (Tomović et al., **2017**). Rudy (**2009**) determined concentrations of Pb, Cd, Hg and As in the *longissimus dorsi* muscle of sheep at various ages. The concentration of these elements increased with age, another influencing factor, except for As that was undetected. Throughout the years, elements such as Ni have gained a new relevance. Indeed, it is now known that Ni has essential functions in the metabolism of nucleic acids (McDonald et al., **2011**). No differences were found for neither effect on Ni concentration.

3.2 Liver mineral status

Liver mineral status for macro and micro minerals is presented in Tables 4 and 5, respectively, and for other elements in Table 6. Copper concentration in the liver of Dorper lambs was 1.9–5.3 times higher than in Damara and Australian Merinos respectively. Higher concentration of Cu in the liver as opposed to the muscle was expected since the liver accounts for half of the Cu present in the organism of animals (Grace & Clark, **1991**). However, this breed's difference was quite interesting as genetic influences have been previously reported on Cu metabolism of sheep breeds. Interestingly, growing Dorpers had less Cu (3.83 mg Cu/kg DM) in the muscle than their Australian Merino (5.64 mg Cu/kg DM) and Damara (5.46 mg Cu/kg DM) counterparts. Wiener (**1979**) reviewed the initial aspects in which genetics were thought to impact upon the mineral metabolism of animals, with a special focus in sheep. According to this author, flocks of different sheep breeds, when reared together, demonstrated different enzootic ataxia (swayback) occurrence. Also, sheep breeds have differences in susceptibility to copper toxicity. Hence, perhaps Merino and Damara are more susceptible to it, thus downregulating Cu accumulation in tissues when present in sufficient concentration. Woolliams, Suttle, Wiener, Field & Woolliams, (**1983**) performed a trial in which three different breeds of sheep were tested for their ability to accumulate and deplete Cu from their organism. In this trial, the authors found that the three breeds, Scottish Blackface, Welsh Mountain and a cross between the first two, had different initial Cu concentration in the liver, prior to a period where their feed was supplemented. When Cu supplementation stopped, Cu depletion rates were noticeable in Blackface sheep. The latter breed reached a threshold of Cu concentration where it was no longer able to accumulate this element. This reality might also be inherent to the Australian Merino and Damara breeds. An interaction between Cu and other dietary minerals might also disturb Cu absorption via the formation of insoluble compounds in the rumen—thiomolybdates for example (Spears, **2003**). This was not considered since all individuals were fed on the same feed in the present study.

TABLE 4. Macromineral concentration (mg/kg dry matter) in the liver of lambs from three different sheep breeds (Australian Merino, Damara and Dorper): growth and restricted

Mineral	Australian Merino		Damara		Dorper		*SEM*	*p* Value (breed)	*p* V (fee leve
	Restricted	Growth	Restricted	Growth	Restricted	Growth			
Calcium (Ca)	252.43	236.06	246.04	235.08	230.55	258.45	5.42	NS	NS
Potassium (K)	9,738.75	9,957.83	9,879.74	9,996.79	9,442.84	10,162.28	73.25	NS	*
Magnesium (Mg)	641.87	646.22	630.56	647.32	605.87	623.33	4.14	**	NS
Sodium (Na)	2,717.92	2,515.98	2,544.80	2,487.85	2,978.85	2,977.10	49.82	**	NS
Phosphorous (P)	11,552.36	11,047.60	10,960.24	10,696.24	10,758.69	10,624.03	60.84	**	**
Sulphur (S)	6,745.62	7,025.25	7,058.00	7,313.00	6,909.86	7,351.90	52.21	*	**

- Abbreviation: NS, non-significant.
- * $p < .05$; ** $p < .01$.

TABLE 5. Micromineral concentration (mg/kg dry matter) in the liver of lambs from 3 different sheep breeds (Australian Merino, Damara and Dorper): growth and restricted

Mineral	Australian Merino		Damara		Dorper		*SEM*	*p* Value (breed)	*p* Value (feeding level)
	Restricted	Growth	Restricted	Growth	Restricted	Growth			
Cobalt (Co)	0.72	0.42	0.64	0.46	0.68	0.49	0.02	NS	**
Copper (Cu)	42.90	45.39	97.05	45.91	226.13	192.08	11.46	**	*
Iron (Fe)	674.55[a]	339.63[b]	515.94[c]	318.50[b]	627.21[a]	296.20[b]	22.11	*	**
Manganese (Mn)	9.60	8.51	9.86	10.00	10.69	9.70	0.19	*	NS
Selenium (Se)	1.24	1.13	1.29	1.30	1.35	1.59	0.04	*	NS
Zinc (Zn)	146.22	124.24	131.70	122.43	147.86	131.20	2.41	NS	**

Note

- Values with different superscripts indicate significant differences when the interaction is significant ($p < .05$).
- Abbreviation: NS, non-significant.
- * $p < .05$; ** $p < .01$.

TABLE 6. Other element concentration (mg/kg dry matter) in the liver of lambs from 3 different sheep breeds (Australian Merino, Damara and Dorper): growth and restricted

Mineral	Australian Merino		Damara		Dorper		*SEM*	*p* Value (breed)	*p* Value (feeding level)
	Restricted	Growth	Restricted	Growth	Restricted	Growth			
Arsenic (As)	1.24[ac]	1.30[ac]	1.25[ac]	1.06[a]	1.41[c]	1.78[b]	0.05	**	NS
Barium (Ba)	2.44	1.97	1.91	2.06	2.54	2.84	0.11	*	NS
Cadmium (Cd)	0.43	0.31	0.71	0.47	0.55	0.44	0.02	**	**
Chromium (Cr)	2.18	1.64	1.84	1.52	2.03	1.55	0.08	NS	**
Lithium (Li)	0.55	0.28	0.47	0.44	0.49	0.88	0.06	NS	NS
Nickel (Ni)	1.54	1.73	1.74	1.58	1.88	2.55	0.14	NS	NS
Lead (Pb)	1.37	1.00	1.15	1.02	1.18	1.17	0.05	NS	NS
Tin (Sn)	2.06[ab]	1.81[a]	1.13[a]	1.22[a]	1.60[a]	3.05[b]	0.16	**	NS
Vanadium (V)	0.32	0.29	0.58	0.29	0.43	0.41	0.04	NS	NS

Note

- Values with different superscripts indicate significant differences when the interaction is significant ($p < .05$).

- Abbreviation: NS, non-significant.
- * $p < .05$; ** $p < .01$.

Iron concentration in the liver was higher in restricted groups, regardless of breed. The interaction of both factors was statistically significant. This difference would suggest higher abundance of Fe-carrying proteins in the liver; however, this has not been reported by Miller et al., (**2019**) using a proteomics approach. Instead, this difference may have two explanations: (a) higher mobilization of Fe in growing lambs and (b) endogenous adipose tissue depletion in the liver, similarly to what occurred in the muscle. Indeed, other elements had common aspects to muscle, namely Co for example, where restricted Australian Merinos had 0.72 mg Co/kg DM liver in comparison to growing lambs which had 0.42 mg Co/kg DM. Moreover, it is important to point out that Fe is a co-factor of aconitase, an enzyme that partakes in gluconeogenesis from the catabolism of amino acids (Campos, **2009**; D'Mello, **2003**). The contrast between groups indicates Fe transport from the muscle to the liver, where its increased availability enhances gluconeogenesis via activation of this enzyme. Further studies are required to confirm this hypothesis.

4 CONCLUSIONS

Seasonal weight loss is indeed a major constraint for ruminant production systems in the tropics. Herein, we studied the mineral profile response of three different breeds to SWL, in muscle and hepatic tissues. Most mineral assessment studies in animal edible tissues comprise similar methodologies without intricate metabolic implications. Indeed, the implications here presented might have a direct relation to factors such as enzymatic activity. Nonetheless, delving further in such matters, using techniques such as the ones used by Van Harten et al. (**2013**) to analyse gene expression of enzymes related to oxidative stress and others not quantified in the mentioned study (e.g. aconitase), would answer this possibility. The different mineral concentrations in these tissues between breeds result from different mineral requirements and response to SWL; hence, the data collected in this trial can contribute to avoid mineral deficiencies of these breeds. Moreover, establishing a routine analysis of minerals in reference tissues can reveal mineral status of animals under SWL. This information could then be used to correct mineral nutrition and avoid production losses.

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CONFLICT OF INTEREST

None.

ANIMAL WELFARE STATEMENT

Australian and European Union guidelines and legislation on care, use and handling of experimental animals were followed. The authors confirm that the ethical policies of the journal, as noted on the journal's author guidelines page, have been adhered to.