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Macrominerals, trace elements and hem and non–hem iron status in muscle *Longissimus dorsi*, from five double purpose lambs breed reared on pasture system in Uruguay

Macrominerales, minerales traza y estado del hierro heme y no heme en músculo *Longissimus dorsi*, de cinco razas de corderos doble propósito criados en sistema de pastoreo en Uruguay

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ABSTRACT

Sheep meat production is facing new challenges, so a thorough knowledge of the attributes of lamb meat produced by different genotypes and under pasture conditions is necessary to characterise these systems, to valorise and differentiate the product from a quality approach and towards a more natural image, attributes that are increasingly taken into account by consumers. This study aimed to characterize the lamb meat nutritionally, coming from five genetic types, reared in a pastoral system, through the content of essential minerals, macro element, Ca, Mg, Na and K, trace elements as Se, Co, Zn, Cu, Mn, total iron (TFe), hem iron (HFe) and non–hem iron (NHFe) and B_{12} vitamin in the *Longissimus dorsi* muscle. The breeds, Corriedale, Merino Dohne, Highlander®, Corriedale Pro, and Australian Merino x Corriedale crossbreed; n=10, were studied. Merino Dohne breed has the highest calcium concentration (66.6 ± 6.3 mg·kg^{-1}), Highlander® and Merino Dohne have a significantly (P<0.05) higher manganese concentration (304.1 ± 26.0 and 308.7 ± 23.6 µg·kg^{-1}, respectively) than the other breeds. There were no significant differences in vitamin B_{12} concentrations between lamb breeds. The HFe and HFe/TFe ratio was higher (P<0.05) in the Corriedale and Corriedale Pro breeds (15.7 ± 0.6 and 15.4 ± 0.7 mg·kg^{-1} and 81.7 ± 2.8% and 76.0 ± 2.2%, respectively) and consequently less NHFe, related to others groups. Also, increased Zn content was obtained in Corriedale (32.6 ± 1.3 mg·kg^{-1}), but other breeds are also rich in zinc. These results show that meat from these breeds qualifies as a good source claim for people with high requirements as children and elders.

Key words: Lamb breeds; meat quality; minerals; haem and no haem iron

RESUMEN

La producción de carne ovina se enfrenta a nuevos desafíos, por lo que el conocimiento profundo de los atributos de la carne de cordero producido por diferentes genotipos y en condiciones de pastura, son necesario para caracterizar estos sistemas, valorizar y diferenciar el producto desde un enfoque de calidad y hacia una imagen más natural, atributos que cada vez más toman en cuenta los consumidores. Este estudio tuvo como objetivo caracterizar nutricionalmente la carne de cordero, proveniente de cinco tipos genéticos, criados en un sistema pastoril, a través del contenido de minerales esenciales; macroelementos, Ca, Mg, Na y K, minerales traza como Se, Co, Zn, Cu, Mn, hierro total (TFe), hierro heme (HFe) y hierro no heme (NHFe) y vitamina B_{12} en el músculo *Longissimus dorsi*. Se estudiaron las razas Corriedale, Merino Dohne, Highlander®, Corriedale Pro y la cruza Merino Australiano x Corriedale; n=10. La raza Merino Dohne tuvo la mayor concentración de calcio (66,6 ± 6,3 mg·kg^{-1}), Highlander® y Merino Dohne tienen una concentración de manganeso significativamente (P<0,05) mayor (304,1 ± 26,0 y 308,7 ± 23,6 µg·kg^{-1}, respectivamente) que las demás razas. No hubo diferencias significativas en las concentraciones de vitamina B_{12} entre las razas de corderos. La relación HFe y HFe/TFe fue mayor (P<0,05) en las razas Corriedale y Corriedale Pro (15,7 ± 0,6 y 15,4 ± 0,7 mg·kg^{-1} y 81,7 ± 2,8% y 76,0 ± 2,2%, respectivamente) y, en consecuencia, menor NHFe, en relación con los otros grupos. También se obtuvo un mayor contenido de Zn en Corriedale (32,6 ±1,3 mg·kg^{-1}), pero las otras razas también son ricas en zinc. Estos resultados demuestran que la carne de cordero de estas razas constituye una buena fuente para personas con altos requerimientos como niños y ancianos.

Palabras clave: Raza de cordero; calidad de carne; minerales; hierro heme y no heme

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INTRODUCTION

Animal protein demand globally, driven by increasing population and discretionary income, is associated with better life quality in great Cities, fast information exchanges, marketing, and cultural evolution [1]. Throughout the World, different animal production system coexists, being lamb production is the most extended and adapted to different Regions, with many other breeds, and also with a predominance of the farm family system [2]. Lamb meat production in Uruguay has undergone different stages in the last five years, with a stable sheep stock of 6.2 million heads [3]. The primary breed reared in Uruguay was Corriedale (42%), a dual-purpose breed, and Australian Merino (26%) for wool production [4].

Due to the variability of external market demand and competitive prices, the Country has recently emphasized increasing the added value of products to be more competitive in international markets. Traditionally, sheep production in the Country carry out in pastoral systems, associated with an image of animal welfare and natural product, whose exploitation is essential for export promotion and to offer healthy food for consumers [5]. A Region in the littoral Northwest of the Country, with abundant high-quality grasses in natural conditions, is traditionally lamb producer, mainly small-scale and predominately Merino for the wool [6]. Red meat is highly nutritional and has a high biological value of protein, bioavailable iron, trace minerals, and vitamins, including a high content of B_{12} [7]. Considering the pastoral-based systems, it observed that the genetic type [8], as well as the type of muscle and the age of the animal [9], impact some nutritional components, which can vary, as well as their oxidative and antioxidant potential [7, 10]. These differences could associate some racial cross, with a specific nutritional composition, to a particular oxidation potential and antioxidant capacity of the meat. Knowledge of the attributes of lamb meat produced by different genotypes in grazing conditions, is necessary to characterize this meat and to add value, take into account the pastoral system, to contribute to human nutrition, consumers demand, and with the objectives of development sustainable (SDO) for this Region of South America. This study aimed to characterize the meat nutritionally, coming from five genetic types, Corriedale, Corriedale Pro, Merino Dohne, Highlander®, and Australian Merino × Corriedale cross breed, reared on a pastoral system, through the content of essential minerals, macro element, Ca, Mg, Na and K, trace elements as, Se, Co, Zn, Cu, Mn and total iron, hem iron and non-hem iron in the *Longissimus dorsi* muscle.

MATERIAL AND METHODS

Animals

The study carries out with lambs from the Experimental Station Mario Alberto Cassinoni (EEMAC) of the Faculty of Agronomy (Udelar) in Paysandú, Uruguay. Five genetic biotypes were studied, Corriedale, Merino Dohne, Highlander®, Corriedale Pro, and Australian Merino x Corriedale crossbreed , n=10 in each group (TABLE I). The lambs were maintained in a single flock for the experiment, grazing forage. A strategic health control of lamb plan followed, and no lamb presented health problems during the study period, and no lamb presented health problems during the study period.

Experimental diets

Lambs were grazing on mixed pasture, including cocksfoot (*Dactylis glomerata*) and white clover (*Trifolium repens*.) (available forage 2,756 kg DM·ha^{-1}) and on a winter annual crops oats (*Avena sativa*) in a rotational grazing with the availability of forage of 2,743 kg DM·ha^{-1}, as shown in TABLE II. To determine the amount of available forage and the types of vegetation present in the grazing area, it was utilized the "Sample Sward-cutting techniques" cutting method and Botanal [11].

TABLE I
Live weight and age at slaughter of lambs for Corriedale, Corriedale Pro, Highlander®, Merino Dohne and a Crossbreed (MA×C; Australian Merino × Corriedale), raising on pastures

Genotypes	Age at slaughter (days)	Live weight (kg)
Corriedale	339.8±4.7	49.3±5.3
Corriedale Pro	343.7±7.3	45.7±2.7
Highlander®	340.4±5.4	53.0±3.6
Merino Dohne	341.3±5.5	55.5±3.8
Crossbreed (MA×C)	334.1±11.2	46.6±8.0

Data represent mean ± SEM of n=10 for each one of genotypes

Muscles samples

At 72 h *post mortem*, the *Longissimus dorsi* muscle was excised from the carcass, subcutaneous fat and silver skin (epimysium) were removed and packed in a vacuum (Vacuum Sealer Machine: Lacor, Model: 69050, Spain) and immediately transported to the laboratory in a cooler with ice packs. The samples were lyophilized (LGJ-12 Freezer Dryer, China) for minerals and non-hem determination. For hem iron analysis, samples were used immediately.

Preparation of solutions and standards

Sub-boiling distilled HNO_3 1 M, prepared with HNO_3 65%, puriss. p.a. (84378, Merck, Germany); HCL 6 M, prepared with HCl 37%, EMSURE, puriss. p.a. (30721, Merck, Germany); $Mg(NO_3)_2$, in 17% HNO_3, magnesium matrix modifier 1% (63043, puriss. p.a. for graphite furnace-AAS, Fluka, Chemika, Switzerland); and $Pd(NO_3)_2$, in 15% HNO_3, palladium nitrate modifier 1% (B0190635, puriss. p.a. for graphite furnace-AAS, Perkin Elmer, Germany) was used for sample preparation and analysis. Millipore-Milli Q distilled deionized water (Merck KGaA, Darmstadt, Germany), with a resistivity of 18 MΩ cm^{-1}, was used throughout. Glassware was soaked for 24 h in dilute (50 mL·L^{-1}) distilled nitric acid and then rinsed thoroughly in distilled deionized water.

Sample Preparation

Samples of pasture (1 g, from a larger sample previously dried to 50°C for 48 h and grounded) and a sample of *Longissimus dorsi* muscle (10 g previously freeze-dried) were dried in a forced-air oven at 105 ± 2°C (Labotecgroup, BJPX-Juneau, Uruguay) until the weight was constant. Subsequently, the samples were ashed in a covered crucible at 550°C in a muffle furnace (Thermolyne, Cimarec 3, USA) with a temperature ramp for 16 h to obtain white residual ash. The ashes were subjected to an acid digestion process in an Erlenmeyer flask, covered with a micro glass ball, with 1 M HNO_3 and 6 M HCl on a hot plate (< 80°C, Thermolyne, 48000 Furnace, USA), then filtered with ashless filter paper (Macherey-Nagel MN 640 d, Germany) and diluted to 10- or 25-mL final volume with distilled deionized water [12]. Blank was also prepared in the same procedure without a sample.

TABLE II

Macro and trace mineral content (mean ± SEM) in the mixed pasture (1) and oat, a winter annual grass (2) offered to five genotypes of lambs for the finishing period

Pastures	Available forage kg·ha^{-1}	Minerals									
		Fe	Zn	Cu	Mn	Se	Co	Ca	Mg	Na	K
		mg·kg^{-1} DM[b]				µg·kg^{-1} DM		g·100 g^{-1} DM			
Pasture 1											
Dactylis glomerata[a]	2,756	225.01±50.72	10.45±0.36	14.41±0.76	114.11±21.71	96.15±13.37	49.40±4.12	0.41±0.03	0.15±0.01	0.06±0.00	1.22±0.01
Trifolium repens[a]		173.30±25.77	12.78±0.47	12.47±2.10	78.86±6.88	46.56±5.38	122.46±14.79	1.34±0.12	0.37±0.01	0.22±0.04	1.32±0.02
Weeds[a]		384.55	18.38	19.30	126.74	39.31	58.97	1.48	0.18	0.10	0.93
Pasture 2											
Avena sativa	2,743	116.02±17.29	6.2±0.58	4.26±0.52	71.70±15.84	74.31±16.66	90.05±9.99	0.35±0.03	0.14±0.01	0.17±0.02	1.23±0.02

[a]Botanical species mainly represented in mixed pasture 1 [b]DM = dry matter

For Se and Co determination in pasture and meat samples, calibration solutions of Se (0, 35, 70, 140, and 280 µg Se·L^{-1}) were prepared immediately before use by dilution (with 0.2% distilled HNO_3 65% in distilled and deionized water) of a 1000 µg Se·L^{-1}, HNO_3 2% standard solution for AA (certified, N93000149, Perkin Elmer, USA). For Co measurements, calibration solutions (0, 5, 10, 20 µg Co·L^{-1}) were prepared immediately before use by dilution (with 0.2% distilled HNO_3 65% in distilled and deionized water) of a 1000 µg Co·L^{-1}, HNO_3 2% standard solution for AA (certified, N93000149, Perkin Elmer, USA).

Se and Co measurements in acidic aqueous dilution (blanks, samples, and calibration curve) were performed with an atomic absorption spectrophotometer (AAS Perkin Elmer, Analyst 300, USA) equipped with a deuterium lamp background corrector, a Perkin Elmer HGA 800, USA, graphite furnace, and a Perkin Elmer AS-800 autosampler, USA [13, 14]. The determinations were conducted using matrix modifiers based on magnesium nitrate and palladium nitrate [15]. Argon (99% purity) was used as a carrier gas, and a selenium or cobalt HCL lamp was used as a light source. All determinations were performed in triplicate.

The detection limit was calculated as three standard deviations of blanks/ average of 10 blanks [15], and precision was calculated as RSD, %, of 10 measures.

Ca, Mg, K, Na, Fe, Zn, Cu, and Mn measurements in acidic aqueous dilution (blanks, samples, and calibration curve) were performed with an atomic absorption spectrophotometer (AAS Perkin Elmer, Analyst 300, USA) with flame, as described Jorhem *et al*. [16]. All samples were analyzed in triplicate.

After extraction with acidified acetone solution, total haem pigments in meat samples were determined as hemin [17]. Hemin was quantified by its absorption peak at 640 nm. Briefly, fresh meat samples (1 g) were finely chopped and macerated in 4.5 mL of 90% acidified acetone in 15 × 90 mm glass test tubes for 1 min on reduced light to minimize pigment fading during the extraction. The tubes were vortexed (ST-100, MRC, Israel), sealed to reduce evaporation, held at room temperature in the darkness for 1 hour then filtered with glass filter paper (Whatman® glass microfiber filters, Grade GF/A, Merck KGaA Germany). The haem iron content was calculated with the factor 0.0882 g iron·g^{-1} hematin. All samples were assayed in duplicate.

The ferrozine method determined the non-haem iron [18]. Briefly, freeze-dried samples of meat (500 mg) were grounded using a mortar and pestle, dissolved in a mixture of 3 mL of 0.1 M citrate phosphate buffer (pH 5.5) and 1 mL of 2% ascorbic acid (as reducing agent) in 0.2 M HCl and left to stand at room temperature for 15 min before adding 2 mL of 11.3% trichloroacetic acid. After centrifugation at 3.000 G for 10 min, the supernatant was recuperated. Reagents were added to 2 mL of the supernatant plus 0.8 mL of 10% ammonium acetate and 0.2 mL of ferrozine, and the absorbance was measured at 560 nm against a standard curve. All samples were assayed in duplicate.

From values obtained for cobalt (Co) content in muscle, vitamin B_{12} was calculated [19].

Statistical analysis

Data of all variables measured were presented as mean ± standard error of the mean (SEM). The normality study of the variables was performed using the Shapiro-Wills test. To determine the effect of breeds on the variables studied, a one-way ANOVA analysis was used for normally distributed variables and Kruskal Wallis test for non-normally distributed variables, following the mathematical model:

$$Y = \mu + Ti + ej$$

where μ: mathematical average, T: treatment relative effect, e: experimental error, Y: response variable (macro and trace minerals, vitamin B_{12}, hem iron and non-hem iron), i: lamb bread, j: random variable

When the lamb breed effect detected a significant difference, *post hoc* means multiple comparisons were realized by the Tukey and Kramer test at $P<0.05$ (normal distributed) and Wilcoxon test with (non-normally distributed).

To determine if it is possible to differentiate meat samples of breed based on their mineral content and gain further insight into the variables that have the most impact on lamb meat, the trace mineral composition dataset and iron forms, such as iron hem, iron non-hem and the ratio iron hem/total iron was included in the principal component analysis (PCA). The statistical analysis was performed with R software [20].

RESULTS AND DISCUSSION

Concerning the mineral composition of mixed pasture and oats (*Avena sativa*) (TABLE II), levels of Ca, Mg, Na, and K could be inadequate for the requirements of different breeds of lambs Masters *et al*. [21], taking into account that levels and bioavailability changes with the season in the Southern Hemisphere Pittaluga [22] and those requirements are not accurate determined in all breeds [21].

Following the requirements of the different genotypes studied here, the pasture's copper content is under the recommended levels according to the lambs under study [23]. Soils in Uruguay are widely deficient in copper, impacting hypocupraemia in cattle (*Bos taurus*) in the littoral west region [24]. However, copper content in plants depends on botanical species, as shown in TABLE II, where cultivated oats are deficient in copper (< 10 mg·kg $^{-1}$ McDowell *et al.* [25]) and native pastures, legumes, and grasses are adequate (> 10 mg·kg $^{-1}$). Also, season and fertilization can affect the level of copper in native grass and cultivated pastures [22, 26]. Suboptimal content in selenium in pasture mixed received in this study was observed previously Guerra *et al.* [6] related to season, particularly in the littoral Region where this study was carried out. Still, it could change with the season, as reported before [6]. For cobalt in pastures fed by lambs, it seems adequate to the requirements of lambs (> 0.1 mg·kg^{-1} Masters *et al.* [21]). Concerning Fe and Mn in pastures, levels were higher than critical levels (> 50 and > 40 mg·kg^{-1}, respectively, McDowell *et al.* [25]), and it is not a problem for lambs. However, for zinc, pastures content is under the critical level of 30 mg·kg^{-1} DM and thus does not fill the requirements of this mineral for growing lamb following the National Research Council recommendation (NRC) [23], but taking into account that relevant differences have breed reported for different breeds [27, 28], caution is due to conclude.

Macrominerals and trace minerals content in *Longissimus dorsi* meat from five genotypes (breed) studied raising on the pasture analyzed shown in TABLES III and IV.

Only Ca showed significant differences (P<0.05) among the macro minerals between breeds. Merino Dohne had the highest value (66.6 ± 6.3 mg·kg^{-1}), and the lowest value was from Corriedale Pro (37.9 ± 5.4 mg·kg^{-1}). Williams [29], Purchas *et al.* [30], and Kasap *et al.* [31] reported Ca values between 4–11 mg·100 g^{-1} of fresh meat. In contrast, Balhaj *et al.* [32] reported much higher values (between 41.96 – 59 mg·100 g^{-1} fresh meat) than those found in this and other studies reported in the literature. This difference may be due to breed studied, muscle type, genre, body weight or feeding intensity Bellof *et al.* [33] since in that work, the lambs, German Merino received barley and mineral salt supplementation from weaning onwards, contrary to our work that lambs were only on pasture.

Animal muscle content calcium is not claimed as a good source for humans but is essential to biochemical function, particularly for muscular fibres contraction [34]. However, it needs to be clarified the minimum content of calcium that muscles need to work and also for meat quality in each lamb breed, and the literature is scarce on this subject.

There were no significant differences in magnesium (Mg) (P>0.05) between the different sheep breeds studied here being similar to values reported by Kasap *et al.* [31] and Hoke *et al.* [35]. There was no significant difference in sodium (Na) and potassium (K), being the values obtained were the same as those reported by Hoke *et al.* [35], Williams *et al.* [29], while Kasap *et al.* [31] said lower values than in this work.

Meat is a good source of vitamin B_{12} for humans [34]. The synthesis depends on cobalt, as the primary component of vitamin B_{12}, either as a cofactor for enzymes that require the vitamin or for microorganisms that synthesize the vitamin as a secondary metabolite. Ruminants need cobalt to provide to the rumen population of methanogenic bacteria synthesizing vitamin B_{12} [36, 37]. Vitamin B_{12} has a molar mass of 1,355 g·mol^{-1} Suttle [38] and contains 4.4% cobalt [19, 38]. Based on this ratio, the value of vitamin B_{12} was estimated in this study (TABLE III). There was no significant difference (P>0.05) between sheep breeds in the concentration of cobalt and vitamin B_{12}. The concentrations obtained in this study are slightly lower than those reported by Juárez *et al.* [39] (0.6–2.5 µg·100g^{-1}). In sheep efficiency of incorporation of cobalt in the molecule of vitamin B_{12} is lower than in bovine animals [37].

Copper, zinc, iron, and manganese are cofactors in several enzymes and contribute to the functioning of the immune system [40]. Iron is present in myoglobin and haemoglobin, proteins responsible for oxygen transport in the blood [41].

Highlander® and Dohne Merino sheep breeds have significantly higher manganese concentrations (304.1 ± 26.0 and 308.7 ± 23.6 µg·kg^{-1}, respectively) (P<0.05). These breeds show high values (60%) concerning those presented by the crossbreed (MA x C), although they were not statistically significantly different from Corriedale. Only some studies are reporting Mn values in lambs. Studies by Hoke *et al* [35] report values of 14 µg.100g^{-1} of lean meat for the Loin Chop cut.

There was no difference in copper levels between different sheep breeds of lambs, even though copper was at a critical level in the pasture. Copper concentrations were similar to those of Lombardi–Boccia *et al.* [42] and Juárez *et al.* [39]. Selenium in the meat of five breeds is adequate for nutrition children, ranged of 76.7 µg·kg^{-1} to 91.1 µg·kg^{-1}. Indeed, a 100 grams of this raw lamb meat has a contribution of 20–25% of RDA [14].

TABLE III

Macro, trace mineral, and calculated vitamin B_{12}[(a)], in raw *Longissimus dorsi* from lamb's breeds (Corriedale, Corriedale Pro, Highlander®, Merino Dohne) and one crossbreed (Australian Merino × Corriedale, MAxC) double purposes reared on pasture

Genotypes	Minerals									Vitamin
	Ca	Mg	Na	K	Zn	Cu	Mn	Se	Co	B_{12}
	mg·kg^{-1}						µg·kg^{-1}			ng.g^{-1}
Corriedale	50.5 ± 7.5 ^{ab}	244.7 ± 12.4	719.9 ± 52.7	3,572.1 ± 205.3	32.6 ± 1.3^{a}	1.40 ± 0.13	251.9 ± 34.4^{ab}	91.1 ± 7.3	28.6 ± 3.7	1.26 ± 0.17
Corriedale Pro	37.9 ± 5.4 ^{b}	269.0 ± 3.1	792.3 ± 29.9	3,846.4 ± 51.2	29.6 ± 0.7^{ab}	1.57 ± 0.10	281.9 ± 33.8^{ab}	81.7 ± 3.8	37.5 ± 5.3	1.65 ± 0.23
Highlander®	64.1 ± 7.0^{ab}	262.9 ± 2.7	694.7 ± 29.9	3,826.5 ± 54.6	30.9 ± 0.9^{ab}	1.50 ± 0.09	304.1 ± 26.0^{a}	86.2 ± 6.7	26.8 ± 3.7	1.18 ± 0.17
Merino Dohne	66.6 ± 6.3^{a}	258.1 ± 6.8	708.6 ± 31.1	3,689.3 ± 86.5	31.4 ± 0.9^{ab}	1.46 ± 0.14	308.7 ± 23.6^{a}	92.2 ± 2.8	29.1 ± 3.5	1.28 ± 0.15
Crossbreed (MA×C)	44.2 ± 6.7^{ab}	258.4 ± 4.1	669.3 ± 32.1	3,569.4 ± 60.4	27.9 ± 0.7^{b}	1.46 ± 0.09	191.5 ± 14.7^{b}	76.7 ± 7.9	34.5 ± 3.8	1.52 ± 0.17
P–value	0.01	n.s.	n.s.	n.s	0.03	n.s.	0.027	n.s.	n.s.	n.s.

[(a)]Calculated in base to Girard *et al.* (2009). Data are presented as mean ± SEM of n=10. [a,b] Data in each column with different lowercase letters show a significant difference between breeds or crossbreed, P<0.05

TABLE IV
Moisture, ashes content, total iron (TFe), hem (HFe) and non–hem iron (NHFe) and the ratio HFe/TFe (%), in raw *Longissimus dorsi* from lamb's breeds (Corriedale, Corriedale Pro, Highlander®, Merino Dohne) and one crossbreed (Merino Australian × Corriedale, MA×C) double purposes reared on pasture

Genotypes	Moisture	Ashes	TFe	HFe	NH Fe	HFe/TFe
	g.100 g^{-1}		mg·kg^{-1}			
Corriedale	75.6±2.7	0.90±0.01	19.5±0.9	15.7±0.6^{a}	3.6±0.6^{b}	81.7±2.8^{a}
Corriedale Pro	75.3±2.9	0.85±0.02	20.2±0.6	15.4±0.7^{ab}	4.6±0.5^{b}	76.0±2.2^{ab}
Highlander®	74.9±2.6	0.85±0.02	20.2±1.9	13.7±0.4^{ab}	6.4±0.5^{a}	68.2±2.1^{b}
Merino Dohne	75.1±2.8	0.87±0.02	20.5±0.6	13.3±0.6^{b}	7.1±0.9^{a}	65.7±3.7^{b}
Crossbreed (MA×C)	75.2±2.8	0.87±0.02	20.7±0.8	14.5±0.7^{ab}	6.3±0.3^{a}	70.2±1.2^{b}
P–value	n.s	n.s.	n.s	0.027	0.001	0.001

Data are presented as mean SEM ± of n=10. ^{a,b} Data in each column with different lowercase letter show significant difference between breeds or crossbreed, $P<0.05$.

The *Longissimus dorsi* muscle of the Corriedale sheep breed presented a higher concentration of zinc (32.6±1.3 mg·kg^{-1}) than the other sheep breeds and crossbreeds ($P<0.05$). Australian studies across different lamb production systems reported zinc levels on average of 2.43 mg.100 g^{-1} of muscle Mortimer *et al.* [43], Pannier *et al.* [44], 34% lower than in the present investigation. Zinc is many Countries' second most deficient mineral [45]. Its role has been actualized related to antiviral immunity Read *et al.* [46], and this meat contributes mainly to the recommendations to protect children and older people Saadoun *et al.* [47] by increasing immune defences, among other beneficial effects.

Corriedale and Corriedale Pro sheep breeds show significantly more Hem Fe and a higher ratio of HFe/TFe, but no difference for Total Fe was obtained (TABLE IV). These results are interesting because iron is a relevant mineral in human nutrition, particularly in children, pregnant women, and adolescents [48]. Iron dietary deficiency is a severe health problem affecting 20–39% of children worldwide (data from World Health Organization (WHO) [49]). The diet has two types of iron: hem iron, derived from haemoglobin, and myoglobin, which represents a small fraction of total iron and is well absorbed, and non–hem iron, inorganic iron with low availability. Hem iron provide by animal proteins such as meat [41].

There was no statistical difference in total iron (TFe) concentration between the five breeds (TABLE IV). Pannier *et al.* [50], in a study based on 2,000 lambs and three main genotypes, did not observe differences in TFe but the difference between Zn levels was observed in different sheep breeds. This response coincides with that obtained in our work. On the other hand, there was a significant difference ($P<0.05$) in the content of hem iron (HFe) and the ratio HFe/TFe, between sheep breeds. Corriedale was the sheep breed with the highest hem iron (HFe) 15.7±0.6 mg·kg^{-1} and HFe/TFe (81.7%) than Merino Dohne sheep breed (13.3±0.6 mg·kg^{-1} and 65.7%). Although the difference in absolute value is 2 mg between the two breeds, this represents a difference of 15%, so it can be considered an important difference. As Hem iron is part of myoglobin, and the concentration of myoglobin depends on the type of muscle fiber, being higher in oxidative type fiber (Type I and IIa), red fibers, and lower in glycolytic type fiber (Type IIx, IIb) in white muscle fiber [51, 52, 53]. A possible explanation is that sheep breeds studied here present differences in fiber type at the same slaughter age [53]. Cottle [54] reported that the Merino Dohne sheep breed could have fibers type IIb (IIx) related to a leaner carcass; consequently, a lower myoglobin content is possible and, therefore, less hem iron. Previous studies by Pannier *et al.* [44] reported a positive association between aerobic markers and mineral content as iron but in lesser magnitude with zinc.

Principal component analysis of meat quality

Principal component analysis (PCA) does carry out to show the relationships among meat trace minerals and iron forms of five breeds produced in Uruguay (FIG. 1). The result of this analysis indicates the genotype effect influenced the majority of the parameters. The PCA makes it possible to visualize a large number on a single graph and estimate the statistical links between the studied individuals.

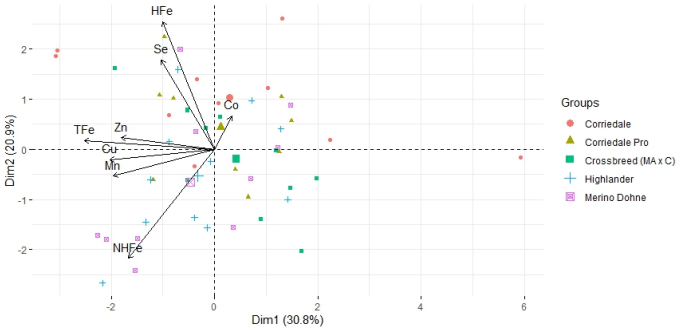

FIGURE 1. Map of selected minerals trace and iron forms of lamb meat from five lamb breeds and cross–breed groups reared on pasture

The loading values for principal component one (PC1) show a strong and positive association with TFe (0.833), Cu (0.670), Mn (0.649) and Zn (0.598). Loading values in PC2 are high and positive for HFe (0.844), and Se (0.591) and negative for NHFe (-0.718). In summary, the variables that best represent PC1 are TFe, (28.14%) and Cu (18.23%), while HFe (42.58%) and NHFe (30.86%) are the variables that contribute most to PC2. Co is the mineral trace that is poorly represented for both principal components.

The individual projection shows no clear discrimination between the breed studied. However, an association can be observed between the Merino Donhe and Hightlander breeds. These breeds in turn have a high association with the mineral HNFe.

Based on PCA statistical analysis, we identified variations in the trace mineral content of the meat among the different animals studied. If the principal components PC1 and PC2 are analysed together, TFe and its components (HFe and NHFe) are the variables that most account for the original variability. All in all, however, there is considerable heterogeneity of distribution or variability of dispersion of the genotypes studied (which somewhat lowers the level of precision, reliability or accuracy of the groups formed).

Meat mineral trace content, principally iron and iron components raised on pastures, is an excellent way to satisfy the requirements of human people. If one wants to increase the value of the meat market, it is crucial to consider the differences between sheep breeds, especially those bred for both wool and meat production.

CONCLUSION

In the context of the study, the genetic component would not be a determining differential for the content of macrominerals and trace elements.

The sheep genotype studied showed a significant association with the minerals Mn, Ca, and Zn, as well as the quantities of HFe and NHFe.

This study justifies and gives excellent scope for further research on sheep breeds and possible interactions between factors such as age, slaughter weight, different diets, and their effect on meat and wool quality.

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Ethics statement

Animals used in this study were maintained in the facilities and environment of the Experimental Station of the Faculty of Agronomy (Udelar) in Paysandú, Uruguay, following the regulations of the University's ethics committee, the Honorary Commission for Animal Experimentation (CHEA, Udelar). The protocol used (Nº 1401) in this investigation was approved by the Ethical Commission for the Use of Animals (CEUA, CENUR Litoral Norte) following the regulations of the CHEA.

Disclosure statement

The authors declare no conflict of interest.

Data availability statement

Data is available on request from the authors. The data that support the findings of this study are available from the corresponding author, Guerra MH, upon reasonable request

Conflict of interest

The authors declare that they have no conflict of interest.

BIBLIOGRAPHIC REFERENCES

[1] Ponnampalam EN, Kerr MG, Butler KL, Cottrell JJ, Dunshea FR, Jacobs JL. Filling the out of season gaps for lamb and hogget production: Diet and genetic influence on carcass yield, carcass composition and retail value of meat. Meat Sci. [Internet]. 2019; 148:156–163. doi: https://doi.org/k8jz

[2] Food and Agriculture Organization of the United Nations. The state of the world's animal genetic resources for food and agriculture. [Internet]. Rischkowsky B, Pilling D, editors. Roma: FAO; 2010 [cited 22 April 2021]; p. 154–155. Available in: https://bit.ly/3u0rMHg.

[3] Dirección de Estadística Agropecuarias (DIEA). Anuario Estadístico Agropecuario 2019. [Internet]. Montevideo, Uruguay: Ministerio de Ganadería, Agricultura y Pesca. 2019 [cited 12 Aug. 2020]: p. 58–61. Available in: https://bit.ly/3RED62I.

[4] Instituto Nacional de Carnes (INAC). Informe Estadístico Año Agrícola 2018–2019. [Internet]. Montevideo, Uruguay: INAC. 2019 [cited 12 Aug. 2023]; p. 25–34. Available in: https://bit.ly/4atpj6a.

[5] Montossi F, Font-Furnols M, del Campo M, San Julián R, Brito G, Sañudo C. Sustainable sheep production and consumer preference trends: Compatibilities, contradictions, and unresolved dilemmas. Meat Sci. [Internet]. 2013; 95(4):772–789. doi: https://doi.org/gmg8v7

[6] Guerra MH, Cabrera MC, Abella DF, Saadoun A, Burton A. Se and I status in pregnant ewes from a pastoral system and the effect of supplementation with Se and I or only Se on wool quality of lambs. Heliyon. [Internet]. 2019; 5(9):e02486. doi: https://doi.org/k8j5

[7] Cabrera MC, Saadoun A. An overview of the nutritional value of beef and lamb meat from South America. Meat Sci. [Internet]. 2014; 98(3):435–444. doi: https://doi.org/gjqwxh

[8] Fernandes-Júnior G, Lôbo R, Madruga M, Lôbo A, Vieira L, Facó O. Genotype effect on carcass and meat quality of lambs finished in irrigated pastures in the semiarid Northeastern Brazil. Arq. Bras. Med. Vet. Zoot. [Internet]. 2013; 65(4):1208–1216. doi: https://doi.org/k8j6

[9] Jacob RH, Pethick DW. Animal factors affecting the meat quality of Australian lamb meat. [Internet] Meat Sci. 2014; 96(2):1120–1123. doi: https://doi.org/f5n8n3

[10] Cabrera MC, Castaño M, Terevinto A, del Puerto M, Saadoun A. Minerals, Heme Iron and Lipid Oxidation in Fresh and Aged Corriedale Lamb Meat from Pasture Based Production System in Uruguay. In: Troy D, McDonnell C, Hinds L, Kerry J, editors. Nurturing Locally, Growing Globally. 63rd International Congress of Meat Science and Technology; 2017 August 13–18th; Cork, Ireland. Wageningen, Netherlands: Wageningen Academic Publishers; 2017; p. 979–980.

[11] Lantinga EA, Neuteboom J, Meijs J. Sward Methods. In: Penning P, editor. Herbage Intake Handbook. 2th ed. Dunston, UK: The British Grassland Society; 2004. p. 24–52.

[12] Shen K, Zhang N, Yang X, Li Z, Zhang Y, Zhou T. Dry Ashing Preparation of (Quasi)solid Samples for the Determination of Inorganic Elements by Atomic/Mass Spectrometry. Appl. Spectrosc. Rev. [Internet]. 2015; 50(4):304–331. doi: https://doi.org/gn8zsd

[13] Stephan CH, Fournier M, Brousseau P, Sauvé S. Graphite furnace atomic absorption spectrometry as a routine method for the quantification of beryllium in blood and serum. Chem. Cent. J. [Internet]. 2008; 2(1):14. doi: https://doi.org/cf2zwx

[14] Cabrera MC, Ramos A, Saadoun A, Brito G. Selenium, copper, zinc, iron and manganese content of seven meat cuts from Hereford and Braford steers fed pasture in Uruguay. Meat Sci. [Internet]. 2010; 84(3):518–528. doi: https://doi.org/crnm26

[15] Butcher DJ. Recent highlights in graphite furnace atomic absorption spectrometry. Appl. Spectrosc. Reviews. [Internet]. 2017; 52(9):755–773. doi: https://doi.org/f5h6r9

[16] Jorhem L. Determination of metals in foods by atomic absorption spectrometry after dry ashing: NMKL Collaborative Study. J. AOAC. Intern. [Internet] 2000; 83(5):1204–1211. doi: https://doi.org/gnv7c5

[17] Hornsey HC. The colour of cooked cured pork. I.—Estimation of the Nitric Oxide–Haem Pigments. J. Sci. Food Agric. [Internet]. 1956; 7(8):534–540. doi: https://doi.org/d4xxvz

[18] Purchas RW, Simcock DC, Knight TW, Wilkinson BHP. Variation in the form of iron in beef and lamb meat and losses of iron during cooking and storage. Intern. Food Sci. Technol. [Internet]. 2003; 38(7):827. doi: https://doi.org/drx65r

[19] Girard CL, Santschi DE, Stabler SP, Allen RH. Apparent ruminal synthesis and intestinal disappearance of vitamin B12 and its analogs in dairy cows. J. Dairy Sci. [Internet]. 2009; 92(9):4524–4529. doi: https://doi.org/d33bqc

[20] R Core Team. R: A Language and Environment for Statistical Computing. Version 4.3.1. Vienna, Austria: Foundation for Statistical Computing. 2023; 1639 p. Available in: https://www.R-project.org/.

[21] Masters DG, Norman HC, Thomas DT. Minerals in pastures – are we meeting the needs of livestock? Crop Past. Sci. [Internet]. 2019; 70(12):1184–1195. doi: https://doi.org/k8s5

[22] Pittaluga-Rossi, A. Minerales en campo natural: variación estacional y por sitio geográfico del contenido de fósforo, cobre, manganeso, zinc, hierro y selenio. [master's tesis on the Internet]. Montevideo, Uruguay: Universidad de la República; 2018 [cited 22 Aug. 2021]; 78 p. Available in: https://bit.ly/3RfIXtu.

[23] National Research Council (NRC). Minerals. In: Nutrient Requirements of Small Ruminants: Sheep, Goats, Cervids, and New World Camelids. Washington, DC: The National Academies Press. 2007; p 112–149.

[24] Torre MH, Viera I, Facchin G, Kremer E, Baran EJ, Porochin T, DiDonato V, Irigoyen C, Irigoyen J, Saldanha S, Bussi J, Ohanian M, Fuentes J. Incidence of hypocupraemia in cattle in northern Uruguay and its alleviation with an injected Cu–Phenylalanine complex. Livest. Prod. Sci. [Internet]. 2005; 95(1–2):49–56. doi: https://doi.org/cc26ws

[25] McDowell LR, Arthington JD. Minerals for Grazing Ruminants in tropical Regions. 4th ed. Gainesville (FLA), USA: Institute of Food and Agricultural Sciences, University of Florida. 2005; 86 p.

[26] Zhai B, Zhao K, Shen X. Effects of Sulphur Fertilizer on Copper Metabolism in Grazing Tibetan Sheep in Fertilized Pasture. Pol. J. Environ. Stud. [Internet]. 2021; 30(6):5351–5356. doi: https://doi.org/k8s7

[27] Zhang H, Nie HT, Wang Q, Wang ZY, Zhang YL, Guo RH, Wang F. Trace element concentrations and distributions in the main body tissues and the net requirements for maintenance and growth of Dorper × Hu lambs. J. Anim. Sci. [Internet]. 2015; 93(5):2471–2481. doi: https://doi.org/f7hmjf

[28] Zhang H, Nie H, Wang Z, Wang F. The net iron, manganese, copper, and zinc requirements for maintenance and growth of Dorper × Hu ewe lambs. Ital. J. Anim. Sci. [Internet]. 2018; 17(4):941–949. doi: https://doi.org/k8s9

[29] Williams P. Nutritional composition of red meat. Nutr. Diet. [Internet]. 2007; 64(s4):s113–s119. doi: https://doi.org/d2k637

[30] Purchas RW, Wilkinson BHP, Carruthers F, Jackson F. A comparison of the nutrient content of uncooked and cooked lean from New Zealand beef and lamb. J. Food Compos Anal. [Internet]. 2014; 35(2):75–82.doi: https://doi.org/k8tc

[31] Kasap A, Kaić A, Širić I, Antunović Z, Mioč B. Proximate and mineral composition of M. longissimus thoracis et lumborum of suckling lambs from three Croatian indigenous breeds reared in outdoor conditions. Ital. J. Anim. Sci. [Internet]. 2018; 17(2):274–278. doi: https://doi.org/k8td

[32] Belhaj K, Mansouri F, Ben-Moumen A, Sindic M, Fauconnier ML, Boukharta M, Proximate Composition, Amino Acid Profile, and Mineral Content of Four Sheep Meats Reared Extensively in Morocco: A Comparative Study. Sci. World J. [Internet]. 2021; 2021:6633774. doi: https://doi.org/k8th

[33] Bellof G, Most E, Pallauf J. Concentration of Ca, P, Mg, Na and K in muscle, fat and bone tissue of lambs of the breed German Merino Landsheep in the course of the growing period. J. Anim. Physiol Anim. Nutr. (Berl). [Internet]. 2006; 90(9–10):385–393. doi: https://doi.org/bdcvsr

[34] Pereira PM de CC, Vicente AF dos RB. Meat nutritional composition and nutritive role in the human diet. Meat Sci. [Internet]. 2013; 93(3):586–592. doi: https://doi.org/f4kwmr

[35] Hoke IM, Buege DR, Ellefson W, Maly E. Nutrient and Related Food Composition of Exported Australian Lamb Cuts. [Internet]. J. Food. Compos. Anal. [Internet]. 1999; (2):97–109. doi: https://doi.org/bz955t

[36] McDowell LR. Vitamin B12. In: McDowell LR, editor. Vitamins in Animal and Human Nutrition. 2nd Ed. Iowa, USA: Wiley-Blackwell. 2000; p 523–563.

[37] González-Montaña JR, Escalera-Valente F, Alonso AJ, Lomillos JM, Robles R, Alonso ME. Relationship between vitamin B12 and cobalt metabolism in domestic ruminant: An update. Anim. [Internet] 2020; 10(10):1855. doi: https://doi.org/gmmfkv

[38] Suttle NF. Mineral Nutrition of Livestock. 4th. ed. Wallingford, UK: CAB International, 2010; p 223–253.

[39] Juárez M, Lam S, Bohrer BM, Dugan MER, Vahmani P, Aalhus J, Juárez A, López-Campos O, Prieto N, Segura J. Enhancing the Nutritional Value of Red Meat through Genetic and Feeding Strategies. Foods. [Internet]. 2021; 10(4):872. doi: https://doi.org/k8tk

[40] Shen X, Song C. Responses of Chinese Merino Sheep (Junken Type) on Copper-Deprived Natural Pasture. Biol. Trace Elem. Res. [Internet]. 2021; 199:989–995. doi: https://doi.org/k8tm

[41] López MAA, Martos FC. Iron availability: An updated review. Intern. J. Food Sci. Nutr. [Internet]. 2004; 55(8):597–606. doi: https://doi.org/dvdxvv

[42] Lombardi-Boccia G, Lanzi S, Aguzzi A. Aspects of meat quality: Trace elements and B vitamins in raw and cooked meats. J. Food. Compos. Anal. [Internet] 2000; 18(1):39–46. doi: https://doi.org/fts92x

[43] Mortimer SI, van der Werf JHJ, Jacob RH, Hopkins DL, Pannier L, Pearce KL. Genetic parameters for meat quality traits of Australian lamb meat. Meat Sci. [Internet]. 2014; 96(2):1016–1024. doi: https://doi.org/f5n83r

[44] Pannier L, Pethick DW, Boyce MD, Ball AJ, Jacob RH, Gardner GE. Associations of genetic and non-genetic factors with concentrations of iron and zinc in the longissimus muscle of lamb. Meat Sci. [Internet]. 2014; 96(2):1111–1119. doi: https://doi.org/k8tp

[45] Ramos A, Cabrera MC, Saadoun A. Bioaccessibility of Se, Cu, Zn, Mn and Fe, and heme iron content in unaged and aged meat of Hereford and Braford steers fedpasture. Meat Sci. [Internet]. 2012; (91)2:116–124. doi: https://doi.org/fzqndm

[46] Read SA, Obeid S, Ahlenstiel C, Ahlenstiel G. The Role of Zinc in Antiviral Immunity. Adv. Nutr. [Internet] 2019; 10:696–710. doi: https://doi.org/ggqmgr

[47] Saadoun A, Cabrera MC, Terevinto A, Puerto M del, Zaccari F. Nutritional Value of Bovine Meat Produced on Pasture. Ref. Module Food Sci. [Internet]. 2019; 2:189–96. doi: https://doi.org/k8tq

[48] Zeng L, Pei L, Li C, Yan H. Iron Deficiency Anaemia. In: Khan J, editor. Current Topics in Anemia. [Internet]. London, UK: IntechOpen Limited. 2018; p 1–26. doi: https://doi.org/k8tr

[49] World Health Organization. Global Anaemia Estimates in Women of Reproductive Age, by Pregnancy Status, and in Children Aged 6–59 Months. [Internet]. 2021 [cited 6 July 2022]. about 2 p. Available in: https://bit.ly/3uTEuoG.

[50] Pannier L, Ponnampalam EN, Gardner GE, Hopkins DL, Ball AJ, Jacob RH. Prime Australian lamb supplies key nutrients for human health. Anim. Prod. Sci. [Internet]. 2010; 50(11–12):1115–1122. doi: https://doi.org/fxqq9v

[51] Aberle ED, Forres JC, Gerrard DE, Mills EW. Principles of Meat Science. 5th. ed. Dubuque, Iowa, USA: Kendall Hunt Publishing. 2012; p 44–46.

[52] Geesink G, Zerby H. Meat Production. In: Cottle DJ, editor. International Sheep and Wool Handbook. Nottingham, UK: Nottingham University Press. 2010; p 395–406.

[53] Pannier L, Pethick DW, Geesink GH, Ball AJ, Jacob RH, Gardner GE. Intramuscular fat in the longissimus muscle is reduced in lambs from sires selected for leanness. Meat Sci. [Internet] 2014; 96(2):1068–1075. doi: https://doi.org/k8ts

[54] Cottle DJ. World Sheep and Wool Production. In: Cottle DJ, editor. International Sheep and Wool Handbook. Nottingham, UK: Nottingham University Press. 2010; p 1–48.