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Trace Element Concentrations in Beef Cattle Related to the Breed Aptitude

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Abstract

Animal feed has traditionally been supplemented with trace elements at dietary concentrations well above physiological needs. However, environmental concerns have led to calls for better adjustment of mineral supplementation to actual physiological needs and, in this context, consideration of breed-related differences in trace element requirements. The aim of this study was to analyze trace element concentrations in the main breeds used for intensive beef production in northern Spain (Holstein-Friesian [HF], Galician Blonde [GB], and GB × HF cross). Samples of blood, internal organs, and muscle were obtained at slaughter from 10 HF, GB, and GB × HF cross calves in the same feedlot. Overall, trace element concentrations in serum and internal organs were within adequate ranges and did not differ between those of breeds, suggesting that trace mineral supplementation was adequate in all groups. The only exception to this was copper, and hepatic copper concentrations were above adequate levels in all calves. This was particularly evident in the HF calves, and the maximum recommended level for human consumption was exceeded in 90% of these animals. Copper, iron, manganese, selenium, and zinc concentrations in muscle were significantly higher in the HF than those in the GB calves, with intermediate values for the crosses. These breed-related differences in trace element concentrations in the muscle may be related to lower muscle mass and/or higher hepatic activity in the HF (dairy) calves than in GB (beef) calves. As meat is an essential source of highly available trace elements in human diets, breed-related differences in trace element concentrations in meat deserve further investigation.

Keywords Trace elements · Breed · Beef cattle · Intensive systems · Organs

Introduction

Trace minerals are essential for maintaining animal health and productivity [1] and must be provided to livestock at optimal concentrations according to requirements that vary during the production cycle. However, considering individual variability in trace mineral contents of feedstuffs, as well as differences in bioavailability and interactions with other nutrients (mainly other trace elements), recommended supplementation [i.e., 2] includes a plus safety factor to ensure that the average gross demand of the population is met. In practice, this is feasible because, in most cases, dietary trace element concentrations can be formulated with large safety margins so that intake can generally exceed requirements without posing a risk to animal health [3, 4]. However, in recent years, concern has arisen in the EU regarding adjustment of mineral supplementation to actual physiological needs, as large loads of trace elements (mainly Cu and Zn) are occurring in the environment [5, 6]. In order to adjust trace element supplementation more closely to nutritional needs, differences in breed requirements must be considered [7]. Ruminants, particularly sheep, display large interbreed differences in copper needs and copper tolerance [1], and episodes of copper deficiency or excessive hepatic accumulation can occur even when animals receive dietary copper concentrations within the limits outlined in the European legislation [8]. Although Cu metabolism has been studied less extensively in cattle than that in sheep, interbreed differences have likewise emerged. For example, Simmental and Charolais appear to have greater Cu requirements than Aberdeen Angus [9, 10], and Jersey cows accumulate more hepatic Cu than Holstein-Friesians [11]. These differences

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may derive from differences in dietary absorption efficiency [11, 12], biliary excretion [13], or feed intake [11]. Moreover, the higher hepatic copper accumulation observed in the Holstein-Friesian (dairy) breed than in the Galician Blonde (beef) breed in northern Spain when reared intensively for beef production with standard trace element supplementation may be related to lower copper mobilization in the muscle in the dairy breed [14]. Apart from Cu, the effect of breed on other essential trace element metabolism has been scarcely studied, even though a recent study found that Simmental cattle had greater liver manganese concentrations compared with Angus cattle [7].

The aim of the present study was to analyze trace element tissue concentrations in the main cattle breeds used for beef production in northern Spain (Holstein-Friesian [HF], Galician Blonde [GB], and their crosses [GB × HF]) when reared under intensive production with a standard mineral-supplemented diet. We hypothesized that male HF calves used for beef production will require lower levels of trace element supplementation because of a lower muscle mass.

Material and Methods

Animals of Study

Trace element concentrations in serum and tissues were evaluated in HF, GB, and GB × HF cross male calves ($n = 10$ for each breed) when slaughtered at age 10 months in September 2016. All animals were from a bigger experiment ($n = 356$) conducted in a commercial feedlot in NW Spain to evaluate the effect of breed on productive beef performance. Animal were reared under identical conditions and fed ad libitum with a typical beef cattle diet based on a standard trace element-supplemented concentrate feed and barley straw (the mean daily barley straw intake was approximately 1 kg/animal). Details of the ingredients and nutritional composition of the diet are shown in Table 1. Trace element concentrations in the concentrate feed, barley straw, and water were analyzed (see Table 2). The calves were allocated in 30 pens (10 per breed, 12 animals per pen). Feed intake was determined by weighing both each day's ration and orts, which were removed before the fresh supply was served. Sample size was calculated considering data of hepatic copper concentrations (mean and variance) of HF (109 and 1136) and GB (70 and 614) calves (9–10-month old) from a previous experiment [15], assuming a power of 80% and a confidence level of 95%. Animals included in this study (one animal per pen) were randomly selected at slaughter based on their ear identification number.

Sample Collection

Blood samples were collected from the coccygeal vein in heparin-free Vacutainer tubes prior to slaughter. Immediately after slaughter, samples of internal organs (liver, kidney (medulla and cortex), brain, and spleen) and muscle (semitendinosus) were removed from each animal with sterile scalpel blades. The liver, kidney, and spleen weights and carcass performance were recorded (Table 3). All samples were immediately refrigerated and transported to the laboratory. Within 6 h of collection, serum was obtained by centrifugation at 3000*g* for 15 min. Tissues were cleaned of connective tissue and fat and homogenated (by using a laboratory tissue homogenizer). Triplicate aliquots of serum (2 mL) and tissues (ca. 10 g) were stored at − 20 °C pending analysis within a 2-week period.

Table 1 Ingredients and chemical composition of the diets supplied in this study

Ingredient (% DM)	
Corn	30
Barley	22.1
Soybean meal (44% CP)	16.7
Corn gluten feed	6.9
Wheat bran	8
Soybean hulls	10
Molasses	1
Palm oil	1.5
Vitamin/mineral premix[a]	3.2
Sodium bicarbonate	0.6
Chemical composition (% DM)	
Crude protein (CP)	15
Crude fiber (CF)	7.3
Neutral detergent fiber (NDF)	20.8
Acid detergent fiber (ADF)	11.1
Ether extract (EE)	3.5
Starch	31.1
Ash	5.8

[a] Vitamin and mineral premix containing (per kg DM) 10.000 IU vitamin A, 2000 IU vitamin D, 25 mg vitamin E, 0.3 mg Co, 16 mg Cu, 32 mg Fe, 0.5 mg I, 40 mg Mn, 0.1 mg Se, and 32 mg of Zn

Sample Analysis

The serum samples (2 mL) were processed by first adding 2.5 mL of 69% nitric acid (TMA, Hiperpure, PanReac, Spain) for 1 h for a cold digestion and then adding 0.5 mL of hydrogen peroxide 30% *w*/*v* (PanReac, Spain) and placing the samples on a thermostatic block at 120 °C for 60 min to complete the digestion. Two milliliters of Milli-Q ultrapure water was then added and, once cool, the digested samples were diluted to 10 mL with Milli-Q ultrapure water. Tissue samples (liver, kidney, brain, spleen, and muscle) (ca. 2 g) were digested in 5 mL of 69% nitric acid and 2 mL 33% *w*/*v* hydrogen peroxide in a microwave digestion system

Table 2 Trace element concentrations in concentrate feed, barley straw, and water

	Concentrate feed (mg/kg)	Barley straw (mg/kg)	Water (μg/L)
Co	0.40	0.091	0.202
Cr	2.91	0.431	0.164
Cu	22.1	2.09	1.76
Fe	163	61	50.1
Mn	101	193	3.30
Mo	1.12	0.351	0.093
Ni	2.12	0.642	1.67
Se	0.249	0.008	0.269
Zn	102	11.2	91.4

(Milestone, Ethos Plus-2, Italy). Digested samples were transferred to polypropylene sample tubes and diluted to 25 mL with ultrapure water. The concentrations of essential trace elements (Co, Cr, Cu, Fe, Mn, Mo, Ni, Se, and Zn) were determined by inductively coupled plasma mass spectrometry (ICP-MS; VG PQ Excel, Thermo Elemental, USA). All the laboratory glassware and polypropylene tubes were washed in 10% nitric acid overnight and then rinsed several times with ultrapure water before use.

An analytical quality control program was used during the study. Signal intensity values were monitored throughout the analysis and subtracted from the readings for calculation of the final values. The limits of detection (LoD) were calculated as three times the standard deviation of the reagent blanks (Table 4). The limits of quantification, expressed as concentration in the sample, were calculated on the basis of the mean sample weight and volume analyzed. The concentrations of the elements in serum and tissue samples were above the quantification limits. Analytical recoveries were determined from two certified reference materials analyzed together with the samples: Standard Reference Material® 1598a Bovine Serum and 1577c Bovine Liver (National Institute of Standards and Technology, USA). Good consistency between the measured and the certified values was observed (Table 4). To evaluate the precision of the analytical method and the overall method, 12 readings from the same digest and single readings from 12 digest of the same sample were recorded. The relative standard deviation (RSD) of these values was 4.15–7.30% (Co), 5.12–6.98% (Cr), 2.13–4.85% (Cu), 4.17–7.77% (Fe), 5.36–9.63% (Mn), 2.36–6.35% (Mo), 5.68–9.54% (Ni), 1.98–5.36% (Se), and 5.63–7.47% (Zn) for the analytical method and the overall method, respectively.

Statistical Analyses

All statistical analyses were carried out with SPSS for Windows (vs 20.0). The Shapiro-Wilk test was used to determine whether data were normally distributed. Two-way ANOVA and post hoc Tukey tests were used to evaluate the effect of breed (HF, GB, and GB × HF) on performance parameters and trace element concentrations in serum and tissues and data were expressed as mean ± SD. The association between carcass performance and liver weight on trace

Table 3 Zootechnical performance of animal by breed (means ± SD)

	Holstein-Friesian (HF)	GB × HF	Galician Blonde (GB)
Initial live weight (kg)	129 ± 21	133 ± 19	143 ± 28
Final live weight (kg)	401 ± 40	399 ± 31	402 ± 29
Feed intake (kg/day)	8.77^{a}	8.06^{ab}	7.45^{b}
Average daily gain (ADG) (kg/day)	1.62	1.58	1.54
Feed conversion	5.41^{a}	5.10^{ab}	4.84^{b}
Carcass weight (kg)	202 ± 21^{a}	231 ± 22^{ab}	244 ± 22^{b}
Carcass performance (%)	50.4 ± 1.21^{a}	57.9 ± 2.31^{b}	60.7 ± 2.88^{c}
Liver weight (kg)	6.28 ± 0.91^{a}	5.45 ± 0.38^{b}	4.94 ± 0.60^{b}
Kidney weight (kg)	0.571 ± 0.044^{a}	0.488 ± 0.039^{b}	0.417 ± 0.051^{c}
Heart weight (kg)	1.68 ± 0.24^{a}	1.56 ± 0.14^{b}	1.51 ± 0.17^{b}
Spleen weight (kg)	0.822 ± 0.129	0.693 ± 0.113	0.747 ± 0.119

Different superscripts indicate a statistically significant difference between groups; Tukey's test; $p < 0.05$

Table 4 Limits of quantification (LoQ) (μg/L) and results of analysis of the certified reference materials: bovine liver SRM-1577 (mg/kg) and animal serum SRM-1598 (μg/L)

Element	LoQ (μg/L)	Certified Reference Material				
		SRM 1577 (mean ± SD; mg/kg)			SRM 1598 (mean ± SD; μg/L)	
		Certified levels	Analyzed levels	%recovery	Certified levels[a]	Analyzed levels
Co	0.2	0.300 ± 0.018	0.311 ± 0.011	103.7	1.24 ± 0.07	1.09 ± 0.08
Cr	0.1	0.053 ± 0.014	0.051 ± 0.011	96.2	(0.33 ± 0.08)	0.34 ± 0.07
Cu	2.3	275.2 ± 4.6	272.9 ± 12.2	99.2	1580 ± 90	1524 ± 70
Fe	6.1	197.94 ± 0.65	196.07 ± 2.69	99.1	1680 ± 60	1696 ± 80
Mn	1.1	10.46 ± 0.47	10.49 ± 0.23	100.3	1.78 ± 0.33	1.80 ± 0.27
Mo	1.4	3.30 ± 0.13	3.32 ± 0.13	100.6	(5.5 ± 1.0)	5.4 ± 0.9
Ni	0.4	0.0445 ± 0.0092	0.0498 ± 0.0104	111.9	0.94 ± 0.18	0.98 ± 0.13
Se	2.1	2.031 ± 0.045	1.999 ± 0.031	98.4	134.4 ± 5.8	132.2 ± 3.8
Zn	8.9	181.1 ± 1.0	181.4 ± 0.9	100.2	880 ± 24	833 ± 36

[a] In brackets reference and information values

element concentrations was evaluated by correlation analysis (Pearson's coefficient). Differences in effects were considered statistically significant at $p < 0.05$.

Results

Data on breed performance in the calves under study are shown in Table 3. Daily intake (18%) and feed conversion (12%) were significantly higher in HF calves than those in GB, while the values were intermediate in GB × HF crosses. When considering performance parameters at slaughter, carcass weight (21%) and carcass performance (20%) were significantly lower in HF calves than those in GB calves, with GB × HF crosses again yielding intermediate values. Consequently, the internal organs were significantly heavier in HF calves than those in GB and GB × HF crosses.

Serum trace element concentrations in calves in our study are shown in Table 5. Serum trace element concentrations were within the adequate values for this animal species. The most comprehensive literature source is Puls [16] and detailed adequate ranges are given in Table 5; these values are also in accordance to reference values given by Suttle [17] and Herdt and Hoff [18]. No statistically significant differences in element concentrations were observed between breeds, except for manganese, the concentrations of which were significantly higher (47%) in HF calves than those in GB calves, again with intermediate values for the crosses.

Trace element concentrations in internal organs in the calves under study are shown in Fig. 1. Trace element concentrations in the liver (the main organ for trace element metabolism, and consequently the best indicator of trace element status) were adequate [16] and no statistically significant differences among breeds were found. The only exception was copper: hepatic copper concentrations in all calves were above the adequate levels (25–100 mg kg^{-1} fresh weight [16]) and significantly higher (37%) in HF calves (189 ± 38 mg kg^{-1} fresh weight) than those in GB calves (138 ± 28 mg kg^{-1}). Moreover, 90, 70, and 40% of liver samples from respectively HF, GB × HF crosses, and GB calves exceeded the maximum residue limit (MRL) level of copper for liver destined for human consumption (140 mg kg^{-1} fresh weight), proposed by EFSA [19]. On the contrary, statistically significant breed-related differences in trace element concentrations were observed in the muscle (expressed in fresh weight) for most elements (Fig. 1): copper (72%), iron (80%), manganese (72%), selenium (84%), and zinc (49%) concentrations were significantly higher in HF calves than those in GB calves, and intermediate values were always obtained for the crosses. Similar results (data not shown) were observed when trace element concentrations in the muscle were expressed in a dry matter basis (% humidity (mean ± SE) 74.8 ± 0.3 HF, 75.0 ± 0.2 GB, 74.9 ± 0.3 GB × HF crosses). Finally, HF calves showed statistically significant lower molybdenum (29%) and selenium (41%) concentrations in the kidney and chromium (24%) and nickel (48%) in the brain than the GB calves or the crosses.

Correlations between trace element concentrations in the muscle and carcass performance (as a gross indicator of muscle mass) and liver weight (as a gross indicator of metabolic capacity) are shown in Figs 2 and 3 respectively. Significant associations ($p < 0.05$) between copper, iron, manganese, selenium, and zinc and carcass performance (negative, all cases $r > -0.407$) and liver weight (positive, all cases $r > 0.445$) were found.

Table 5 Trace element concentrations in serum in Holstein-Friesian (HF), Galician Blonde (GB), and their crosses (GB × HF)

	HF		GB × HF		GB		Normal range (Puls 1994)
	Mean ± SD	Range	Mean ± SD	Range	Mean ± SD	Range	
Co (μg/L)	1.40 ± 0.03	(1.35–1.44)	1.39 ± 0.03	(1.35–1.45)	1.38 ± 0.03	(1.30–1.45)	0.9–15
Cr (μg/L)	0.274 ± 0.202	(0.239–0.300)	0.298 ± 0.035	(0.249–0.353)	0.289 ± 0.051	(0.187–0.369)	0.25–0.30
Cu (mg/L)	0.786 ± 0.066	(0.703–0.873)	0.773 ± 0.092	(0.616–0.912)	0.812 ± 0.063	(0.713–0.908)	0.6–1.5
Fe (mg/L)	2.11 ± 0.09	(1.93–2.27)	2.10 ± 0.09	(1.98–2.25)	2.18 ± 0.22	(1.93–2.59)	1.3–2.5
Mn (μg/L)	10.98 ± 2.72[a]	(8.75–17.83)	9.29 ± 1.33[ab]	(6.30–11.37)	7.44 ± 2.28[b]	(6.55–10.84)	6–70
Mo (μg/L)	50.4 ± 10.8	(37.1–73.2)	54.5 ± 10.4	(44.3–73.2)	54.3 ± 8.6	(36.1–69.4)	10–100
Ni (μg/L)	3.21 ± 0.28	(2.94–3.44)	3.19 ± 0.22	(2.97–3.33)	3.22 ± 0.06	(3.01–3.32)	1.2–5.6
Se (mg/L)	0.253 ± 0.092	(0.130–0.425)	0.256 ± 0.092	(0.145–0.456)	0.268 ± 0.070	(0.181–0.408)	0.08–0.3
Zn (mg/L)	1.34 ± 0.16	(0.93–1.48)	1.24 ± 0.16	(1.05–1.51)	1.20 ± 0.09	(0.98–1.32)	0.80–1.40

Different superscript letters indicate a significant difference between breeds

Discussion

The results of our study indicate that, with the exception of copper, trace element concentrations in serum and internal organs (being the liver, the main indicator of trace mineral status) were adequate. As previously indicated, the excessive hepatic copper accumulation in cattle under intensive production is a matter of current debate [6] and needs further attention to avoid risks for consumers and animals.

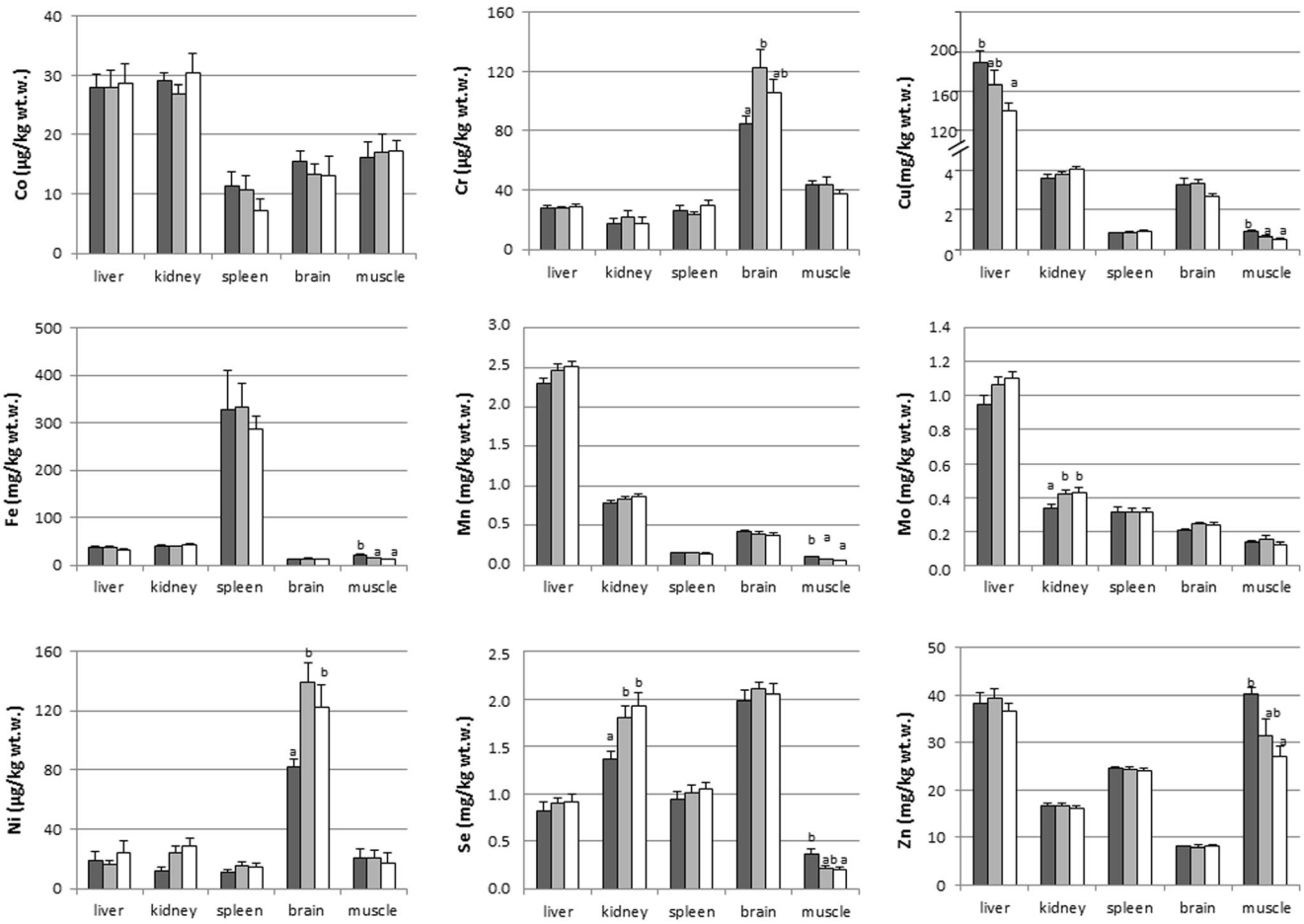

Fig. 1 Trace element concentrations (arithmetic means ± SD) in internal organs (liver, kidney, spleen, brain) and muscle in Holstein-Friesian (HF) (▬), Galician Blonde (GB) (▭), and GB × HF crosses (▭). For the same organ, different letters indicate statistically significant differences between breeds (*indicates a statistically significant difference between groups; Tukey's test; $p < 0.05$)

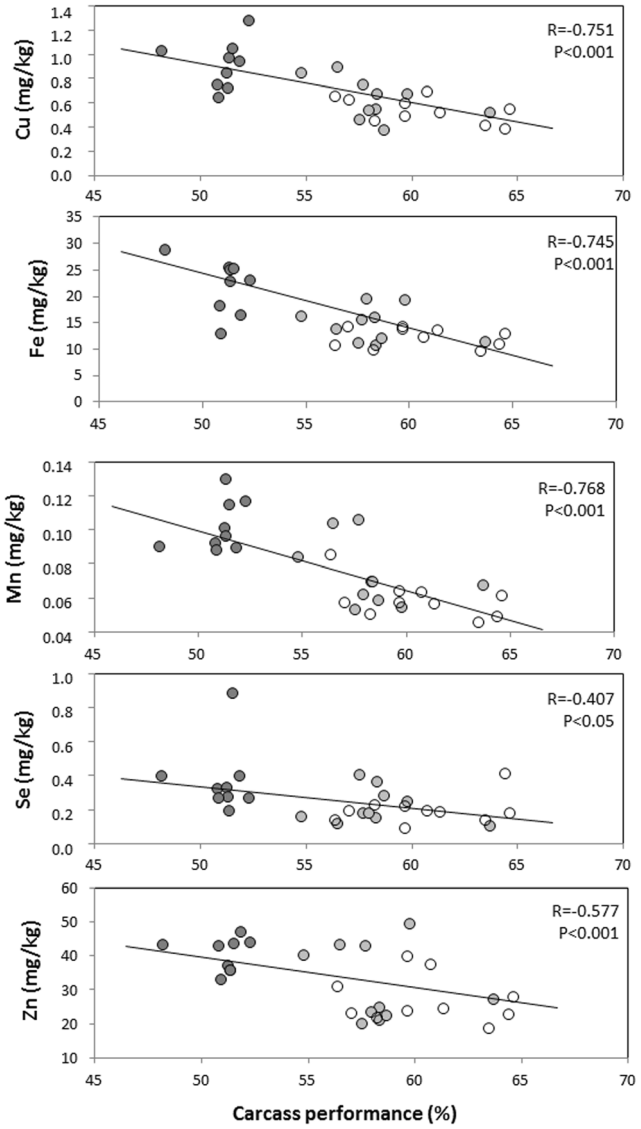

Fig. 2 Scatter plot showing associations between carcass performance (%) and trace element concentrations in muscle samples from the calves under study ((●) Holstein-Friesian (HF), (○) Galician Blonde (GB), (◐) GB × HF crosses)

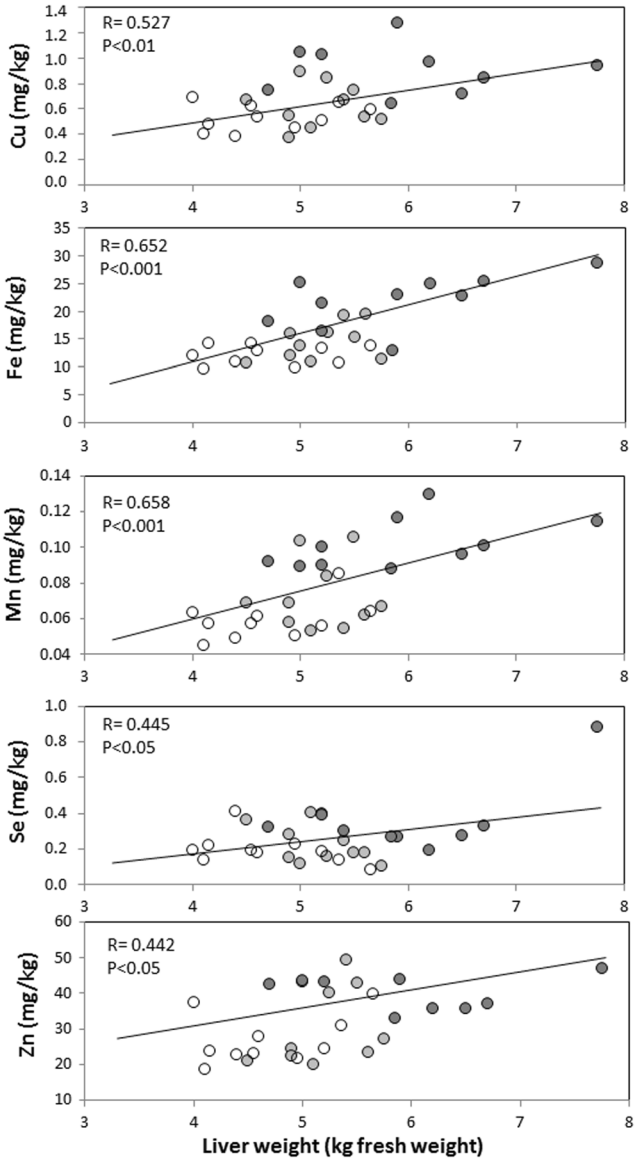

Fig. 3 Scatter plot showing associations between liver weight (kg fresh weight) and trace element concentrations in muscle samples from the calves under study ((●) Holstein-Friesian (HF), (○) Galician Blonde (GB), (◐) GB × HF crosses)

The pattern of trace element distribution found in our study, i.e., no breed-related differences in serum, except manganese, and most tissues (including liver) but higher concentrations in the muscle of HF calves, may be associated to anatomic and metabolic differences between dairy (HF) and beef (GB) aptitude breeds [20]. The same pattern of distribution had been described for Cu in a previous study [14], but as far we are concerned, it is the first time that interbreed-related differences are found for other trace element concentrations in the muscle. Interestingly, although trace element concentrations in the muscle were lower than those in other tissues (1–2 orders of magnitude lower than in the main reservoir; Fig. 1), the total concentrations of trace elements were higher in this tissue than those in the rest of the body due to the large volume of muscle. Even though correlation does not imply causation, the negative associations between carcass performance and trace element concentrations in the muscle may suggest a greater need for mobilization of minerals to the muscle in beef breeds [14]. Trace element concentrations in meat do not depend on the dietary intake but could be related to the metabolic capacity of the animal to deliver trace elements from the liver to the muscle, which could also explain the significantly higher manganese concentrations in the serum of the HF calves compared with GB. It is possible that HF calves have a higher metabolic capacity to deliver trace elements into the muscle. In fact, dairy breeds are known to have a higher hepatic metabolic capacity than beef breeds [21, 22], which is related to the larger livers in

the former [23]. In this respect, trace elements from the milk are essential nutrients for the newborn [24] and it is well demonstrated that trace elements (particularly Zn, Se, and I) have a great role in maintaining immunity and udder health [25].

Breed-related metabolic differences may also explain the statistically significant lower selenium and molybdenum concentrations in the kidneys, and chromium and nickel in the brain of HF calves. Selenium and molybdenum, together with iodine, are renally excreted [17]: they are absorbed in excess in the intestine (proportionally to their concentration in the diet) and their homeostatic regulation mainly occurs in the kidney. This particular type of metabolism makes it feasible to increase the tissue concentrations of elements and has been taken advantage of in the industry to produce iodine- and selenium-enriched animal products [26, 27]. However, although breed-related differences in biliary excretion are well described in cattle for elements such as copper and zinc [13, 28], as far we are aware, no information is available in relation to breed-related differences in renal excretion of elements. Information on chromium and nickel metabolism in cattle is scarce. Both elements are commonly associated in nature [29] and are used in many industrial and anthropogenic processes (i.e., to make dental alloys). Although chromium and nickel have long been considered toxic non-essential elements, recent data indicates that both are generally required in small amounts for normal animal growth and they are therefore now considered essential micronutrients [30, 31]. The role of chromium in glucose metabolism as part of the glucose tolerance factor is especially important (potentiating insulin action and improving glucose utilization, [32]), particularly in the brain, in which chromium alleviates cerebral oxidative stress in diabetes resulting from hyperglycemia [33]. Although it is difficult to discuss the relevance of our findings in view of the lack of information about the metabolism of these elements, it is possible that the breed-related differences in chromium and nickel concentrations in the brain may be at least partly related to differences in glucose metabolism depending on the predominance of anabolic reactions (GB) or catabolic reactions (HF) in the different breeds.

Conclusions

The findings indicate that the standard trace element supplementation used in intensively reared beef cattle is adequate for the main breeds reared in northern Spain, although copper supplementation may be excessive and lead to storage of copper in the liver above the maximum recommended levels, particularly in HF calves. Since meat is an essential source of highly available trace elements in human diets, the significantly higher trace element concentrations in muscle of HF (dairy aptitude) than those in muscle of GB (beef aptitude) deserve further investigation.

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Compliance with Ethical Standards

All the experimental work was conducted in accordance with the European and Spanish legislation on the use of animals for research. All animal used was previously approved by the Bioethical Committee of the University of Santiago de Compostela and animals were enrolled with owner consent.

Conflict of Interest The authors declare that they have no conflict of interest.

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