Essential and toxic trace element concentrations in different commercial veal cuts in Spain

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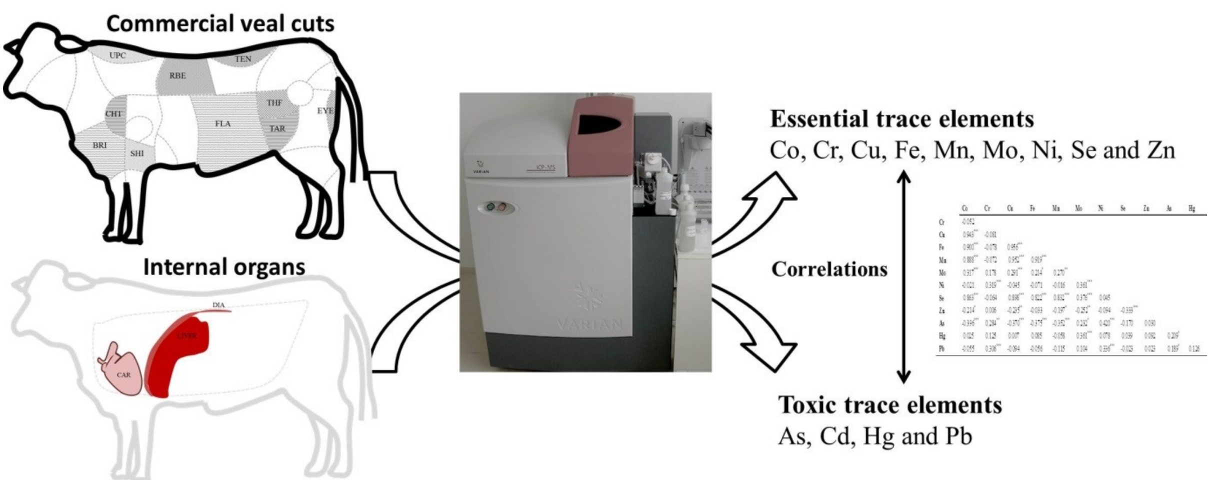

Abstract

The aim of this study was to evaluate essential and toxic element concentration of ten commercially available veal cuts, together with diaphragm, cardiac muscle and liver tissue from 10 animals of "Galician Supreme Veal". Essential trace elements (Co, Cr, Cu, Fe, Mn, Mo, Ni, Se and Zn) and toxic elements (As, Cd, Hg and Pb) were determined by ICP-MS. Essential trace element concentrations ranged from 0.002-55.64 mg/kg between muscles. Toxic element concentrations were very low, and high numbers of samples showed unquantifiable residues of Cd and Pb. Veal cuts including muscles with a high proportion of oxidative slow-twitch fibers (diaphragm and cardiac muscle) showed significantly higher essential trace element concentrations, the lower concentrations being found in veal cuts including glycolytic fast-twitch fibers (eye round). Our results suggest that essential and toxic trace element concentration could be used as a new meat quality parameter, or to add further value to certain products (i.e. livestock reared on extensive systems with high physical activity).

1. Introduction

Meat is an important component of dietary consumption patterns in today's society, with beef and cattle products accounting for a significant portion of the global food market. Consumption rates in Europe were estimated to be over 10.5 kg of beef and cattle meat per capita in 2014 (https://data.oecd.org/agroutput/meat-consumption.htm). Meat and meat products for human consumption have been traditionally evaluated in terms of their proximate macronutrient composition (i.e. moisture, protein and fat contents). Recently, other characteristics such as the fatty acid profiling of meat from different animal species (Enser et al., 1998; Morán et al., 2013; Waszkiewicz-Robak et al., 2015), vitamin composition (i.e. B12) (Szterk, Roszko, Małek, Czerwonka, & Waszkiewicz-Robak, 2012) as well as essential and toxic trace element concentration have gained increased attention due to variation related to factors such as breed, the age of the animal, feeding practices and geographical conditions amongst others (Czerwonka & Szterk, 2015). The accumulation of trace elements is important from a toxicological point of view and lead the European Union to set up maximum limits for certain toxic elements in foodstuff for human consumption (Commission Regulation (EC), No 1881/2006).

To date, studies investigating essential and toxic element concentrations in different animal species have primarily analyzed samples from the liver and kidney, focusing on the relationship between concentrations found in these organs and the health and mineral status of the animals under different production systems (Tomović et al., 2011) or experimental conditions (García-Vaquero, Miranda, Benedito, Blanco-Penedo, & López-Alonso, 2011). The accumulation patterns of trace elements were also described within these organs, analyzing differences in metal accumulation between the medulla and cortex in the kidney (Olsson & Oskarsson, 2001), different accumulation in the lobules in the liver (Miranda et al.,

2010) and histological differences due to variation at hepatic cellular and sub-cellular levels (Garcia-Vaquero, Benedito, Lopez-Alonso, & Miranda, 2012).

There is a notable absence of research investigating essential and toxic trace element accumulation in muscle tissue, the current research is limited to a range of commercial cuts (Czerwonka & Szterk, 2015; Dermauw et al., 2014; García-Vaquero, Miranda, Benedito, et al., 2011; McGilchrist, Greenwood, Pethick, & Gardner, 2016) and non-specified muscular tissue in other instances (Abou-Arab, 2001; Sedki, Lekouch, Gamon, & Pineau, 2003). Recent studies report metal accumulation in treated meat, such as smoked meat (Mitić, Stojković, Pavlović, Tošić, & Mitić, 2012) and other meat products such as sausages and hamburgers (Abedi, Ferdousi, Eskandari, Seyyedahmadian, & Khaksar, 2011; González-Weller et al., 2006).

This study seeks to investigate essential and toxic trace element concentrations in order to develop a complete trace element distribution profile of different commercial cuts of veal, representative of the beef carcass and patterns of human consumption in Spain and many other countries.

2. Material and methods

2.1. Experimental design and sample collection

For this study veal samples from ten male Galician blond calves from Lugo (Galicia, Spain) were selected. Animals were raised under the regulations of the protected geographical indication (PGI) of “Galician Veal” and its meat was certified and labelled as “Supreme Galician Veal” category. “Galician veal” was selected for being one of the main meat production systems in our region and due to its high presence in the national market, with more than 50% of the PGI meat commercialized in Spain certified as “Galician Veal” in the year 2014 (http://www.terneragallega.com/pdf/informe_castellano.pdf). Basically, in this production system calves are suckled on their mothers until slaughter and the diet of the mother is based on local pasture that could be supplemented with fresh forage and grain-based authorized concentrate (Council Regulation (EC), No 510/2006 ; Commission Regulation (EC), No. 2400/1996; Regulation, (EU) 2015/1393).

Samples were collected at slaughter when calves were approximately 9 months old and 242±7 kg carcass weight. Ten muscle cuts were taken from each animal, being representative of the different commercial categories (extra, first A, first B, second and third categories) in Spain (Sañudo & Campo, 1998) (see Fig. 1). The commercial veal cuts selected for this study (rib boneless entrecote (RBE), tenderloin (TEN), eye round (EYE), thick flank (THF), tail of rump (TAR), chuck tender (CHT), shin (SHI), upper chunk (UPC), flank (FLA) and brisket (BRI) are represented in Fig. 1. The muscular fibres constituting the different commercial veal cuts selected are summarized in table 1. In addition, based on previous trace element accumulation studies in calves (Gutiérrez, 2009), samples of diaphragm (DIA) and cardiac muscles (CAR), as well as liver - one of the main target organs of trace element accumulation and mineral status of the animals (Nwude, Okoye, & Babayemi, 2011) - were collected.

All samples of approximately 100 g were collected immediately after slaughter, packed individually and refrigerated at 4-6°C for 2 hours until further processing in the laboratory and then stored at - 18°C for further analysis.

2.2. Chemicals

The following chemicals were purchased for trace element determination of the muscular samples: concentrated nitric acid (65%, Suprapur grade, Merck), hydrogen peroxide (30 % p/v, Sigma-Aldrich) and certified reference material (Standard Reference Material® 1577c Bovine Liver, National Institute of Standards & Technology, USA). Ultra-pure water of resistivity 18 MΩ cm was obtained from a Milli-Q® Plus model (Millipore Co.).

2.3. Sample analysis

Tissue samples of approximately 1 g were digested with nitric acid and hydrogen peroxide in a microwave digestion system (Millestone Ethos Plus) and diluted to 15 mL with ultrapure water following the protocol previously described by García-Vaquero, Miranda, López-Alonso, Castillo, and Benedito (2011). The concentrations of essential (cobalt (Co), chromium (Cr), copper (Cu), iron (Fe), manganese (Mn), molybdenum (Mo), nickel (Ni), selenium (Se) and zinc (Zn)) and toxic (arsenic (As), cadmium (Cd), lead (Pb) and mercury (Hg)) trace elements were determined by inductively coupled plasma mass spectrometry (ICP-MS; VGElemental PlasmaQuad SOption) under the following previously established operational conditions (Gutiérrez, 2009). Briefly, the main analytical parameters consisted on RF power 1.35 kW, plasma flow rate of 14 mL/min, auxiliary gas flow rate 1 mL/min, nebulizer gas flow 0.8 mL/min and 3 readings/replicates.

2.4. Analytical quality control

An analytical quality control was applied throughout the study. Blanks and certified reference material were run alongside samples. Blank values were subtracted from all sample readings and the limits of detection were calculated as three times the standard deviation of the reagent blanks and were based on the mean sample weight analyzed. Non-detectable (ND) concentrations were assigned a value of half the quantification limit. Analytical recoveries were determined from certified reference material (Standard Reference Material® 1577c Bovine Liver, National Institute of Standards & Technology, USA). There was a good agreement between the measured and the certified values (table 2).

2.5. Statistical analysis

All statistical analyses were done in SPSS (v. 20). Normality and equality of variances of the data were analyzed using Kolmogorov-Smirnov and Levene tests respectively and data was logarithmically transformed. One way Anova tests were used to check for differences in essential and toxic trace element concentrations amongst the different muscle samples analysed and post-hoc DHS Tukey tests when needed. Associations between pairs of essential and toxic trace element concentrations in the different muscle samples were evaluated by Spearman correlation analysis. Statistical treatments were not performed in the case of Cd and Pb due to the high number of muscle samples with residues under the detection limit of the method.

3. Results and discussion

Essential trace element concentration (expressed as average ± standard deviation of the mean on a wet weight basis (wt.w.)) in the liver (as the main indicator of trace mineral status) of calves in this study (see table 3) were within the adequate ranges Puls (1994). Toxic metal acumulation in the liver (As: 2.71±0.27 µg/Kg; Cd: 7.88±0.80 µg/Kg; Hg: 1.53±0.05 µg/Kg and Pb: 6.45±1.14 µg/Kg) was low, similar to that found in other non-polluted agricultural areas worldwide (García-Vaquero, López-Alonso, et al., 2011; Nriagu et al., 2009; Yabe et al., 2012). Thus, it is unlikely that trace element deficiencies and/or metal interactions, as well as toxic effects resulting from excessive toxic and trace element accumulation are affecting animals of this study.

Essential trace element concentrations in the different veal cuts, diaphragm and cardiac muscles analysed in our study are presented in table 3. Little information exists in the literature on trace element concentration for specific veal cuts (García-Vaquero, Miranda, Benedito, et al., 2011; McGilchrist et al., 2016; Tomović et al., 2011). Overall, trace element concentrations in veal in our study are within the adequate range of muscle in cattle (in parenthesis adequate ranges (expressed as wt.w.) according to Puls (1994) for Fe (9-21 mg/kg), Se (0.07-0.15 mg/kg) and Zn (20-70 mg/kg); however, no information is given in Puls (1994) for Co and Mo concentrations in muscle, and for Cr (100-200 µg/kg), Cu (1.2-1.5 mg/kg), Mn (0.5-0.9 mg/kg) and Ni (350-400 µg/kg) concentrations measured in our study are below the adequate ranges. Nevertheless, it should be noted that no information is given on how these ranges were determined and only in the case of Zn levels of 20-30 and 70 mg/kg wet weight, were these explicated for light and dark fibre muscles respectively. The trace elements from the adequate ranges in this study, noted above, were those that demonstrated the largest ranges (Cu and Mn) and those lacking empirical explanation within the current literature (Cr and Ni). Overall, the mean trace element concentrations in the muscles investigated for this study were similar to those previously reported (Abou-Arab, 2001; Gawdaman, Sudha, & Pal; McGilchrist et al., 2016; Nwude, Okoye, & Babayemi, 2010; Tomović et al., 2011), although a comprehensive comparison cannot be made for most veal cuts due to disparities related to muscle cuts, age, sex and production systems of the animals in different studies. When compared with the data cumulated in the course of this project, from samples obtained in our region, the trace element concentration observed was consistent with the analysis of muscle pieces in other instances (García-Vaquero, Miranda, Benedito, et al., 2011; Gutiérrez, 2009).

When evaluating trace element concentrations in detail, across the array of veal cuts assessed in our study, statistically significant differences between types of muscles were observed for Co, Cu, Fe, Mn, Se and Zn; elements that play a vital role in muscular metabolism (table 3). No relationship could be established between trace element concentrations and commercial categories. However, with the exception of Zn, certain accumulation patterns were appreciated: the highest trace element concentrations were found in the cardiac muscle (CAR) —up to ca. 3-fold most other veal cuts ($p<0.001$)—followed by diaphragm muscle (DIA)—that also showed statistically significantly higher levels of Cu, Fe and Mn than the rest of the muscle sample—whereas the lowest concentrations were found in the eye round (EYE) (except for Se). This trace element distribution pattern across the carcass seems to be related to the predominant metabolic and contractile activities of the muscles (Talmant, Monin, Briand, Dadet, & Briand, 1986). Trace element concentrations are higher in muscles with a predominance of oxidative slow-twitch fibers or red fibers (Czerwonka & Szterk, 2015), cardiac muscle, masseter and diaphragm represent the best examples in the bovine carcass (Talmant et al., 1986). Slow-twitch muscular fibers contain higher levels of mitochondrial enzymes, myoglobin and iron-containing cytochrome to support an enhanced aerobic metabolic capacity (Choi & Kim, 2009). In contrast to this, trace element concentrations are low in muscles with a high proportion of glycolytic fast-twitch fibers, *Semitendinosus* included in the eye round (EYE) are characteristic of this in the bovine carcass (Talmant et al., 1986). Other veal cuts including predominantly glycolytic muscles according to Talmant et al. (1986) that show low trace element concentrations in our study are tenderloin (*Psoas mayor*), flank (*Rectus abdominalis*), tail of rump (*Tensior fasciae latae*) and brisket (*Pectoralis profundus*).

Meat quality was traditionally defined by its compositional quality (mainly fat and protein) and palatability factors (i.e. visual appearance, smell, firmness, juiciness, tenderness, and flavor) (FAO, 2014). Branding, differentiation by healthiness and by process characteristics (organic production and animal welfare), are contemplated as new possibilities for the development of competitive meat products (Grunert, Bredahl, & Brunsø, 2004). The results of essential trace elements of the present study could be used to add further value to non-competitive meat cuts (i.e. diaphragm and cardiac muscle could be marketed as a source of essential trace elements to population segments suffering trace elements deficiencies) or production systems (i.e. certain livestock breeds reared on extensive systems with high degree of physical activity could show differences in essential trace element profile in the carcass).

Toxic element residues in veal cuts in our study are presented in table 4. Overall metal concentrations were very low and a high proportion of samples had unquantifiable residues (especially of Cd and Pb, 92 and 73% respectively), while the remainder had concentrations very close to the detection limit. These results were similar to or lower than to previous studies on trace element accumulation in meat, both in- and out-side our region (Falandysz, 1993; García-Vaquero, Miranda, Benedito, et al., 2011; Gawdaman et al.; González-Weller et al., 2006; Tomović et al., 2011) and none of the muscle samples exceeded the maximum admissible levels established by the European Commission for meat products (0.05 mg Cd/kg and 0.1 mg Pb/kg wt.w.; (Commission Regulation (EC), No 1881/2006; Commission Regulation (EU), No 488/2014). In the case of As, the European Union has no legal limits in meat products, although recently the European Food Safety Authority (EFSA) was asked to prepare a scientific briefing on human health risks related to the presence of As in foodstuffs (Commission Recommendation (EU), 2015/1381). When analysing toxic element accumulation within the different veal cuts, it was observed that, unlike essential trace element concentrations, the lowest As residues ($p<0.01$) were found in the cardiac muscle whereas the highest residues were measured in veal cuts including muscles with a high proportion of glycolytic fast-twitch fibers. These results are consisting with previous findings (García-Vaquero, Miranda, Benedito, et al., 2011) and could suggest that muscles with higher metabolic activity could have a greater capacity for toxic metal detoxification.

When evaluating the associations between trace elements in the different veal cuts in our study (table 5) strong relationships ($r_s> 0.5$, $p<0.001$ in all cases) were found between the essential elements that showed a common intermuscular distribution pattern (such as Cu, Fe, Mn and Zn). I.e. a strong association between Cu and Fe concentrations in the different samples analysed in this study is shown in Fig. 2. These results could reinforce the hypothesis that under conditions of adequate mineral status as in this study, trace element concentrations in the muscular tissue are dependent on its own internal metabolism. To maintain the mineral homeostasis, in the muscle as well as in other tissues, the organism developed different mechanisms as metallothioneins, chaperones and other metal transporters (Andrews, 2002);

special relevance has acquired the divalent metal transporter 1 (DMT1), involved in the traffic into the cells of divalent metals such as Fe, Zn, Cd, Cu, Co, Ni and Pb (Gunshin et al., 1997; Mackenzie, Takanaga, Hubert, Rolfs, & Hediger, 2007) and ubiquitously expressed in all tissues. The fact that some of the essential metals (Cr, Mo and Zn) do not follow the same intermuscular distribution pattern could be related to metal interactions or antagonisms to maintain a correct mineral balance.

4. Conclusions

Under the conditions of this study, including animals with adequate mineral status, there were significant differences on essential and toxic trace element concentrations across the carcass. Veal cuts including muscles with a high proportion of oxidative slow-twitch fibers (i.e. diaphragm and cardiac muscle) showed higher essential trace element concentrations, the lower concentrations being found in veal cuts including glycolytic fast-twitch fibers (i.e. eye round). Since the proportion of slow and fast twitch fibers in the muscle can be modulated by genetic selection and physical activity, our results suggest that essential and toxic element concentration could be used as a new meat quality parameter or to add further value to certain products (i.e. certain livestock breeds reared on extensive systems with high degree of physical activity). More studies are needed in muscular tissue to further understand and evaluate the importance of trace element accumulation in different veal cuts.

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Fig. 1. Carcass showing the commercial muscle cuts selected. Muscle abbreviations are as follows RBE (rib boneless entrecote), TEN (tenderloin), EYE (eye round), THF (thick flank), TAR (tail of rump), CHT (chuck tender), SHI (shin), UPC (upper chuck), FLA (flank) and BRI (brisket). Commercial categories of veal cuts according to the Spanish regulations are represented in the figure by different filling patterns.

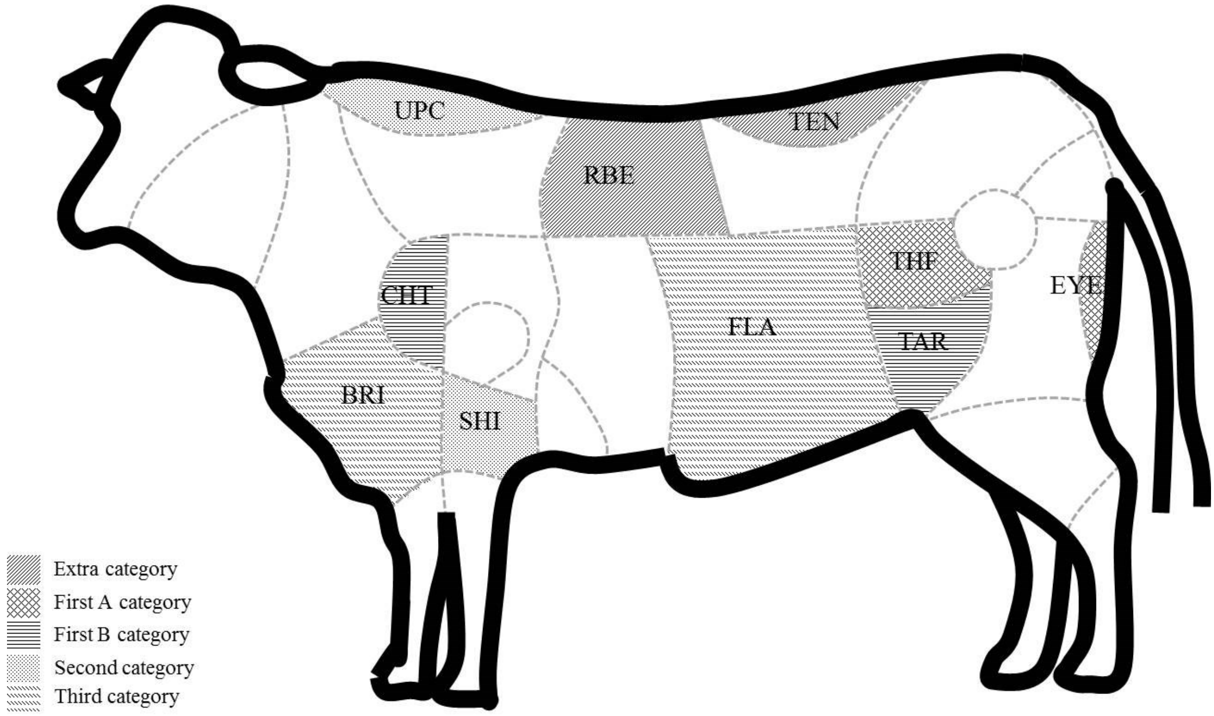

Fig. 2. Scatterplot showing correlation between Cu and Fe concentrations (rs= 0.829, P<0.001, n=120) in the different muscle samples. Abbreviations of muscles were as follows: RBE (rib boneless entrecote), TEN (tenderloin), EYE (eye round), THF (thick flank), TAR (tail of rump), CHT (chuck tender), SHI (shin), UPC (upper chuck), FLA (flank), BRI (brisket), DIA (diaphragm) and CAR (cardiac muscle).

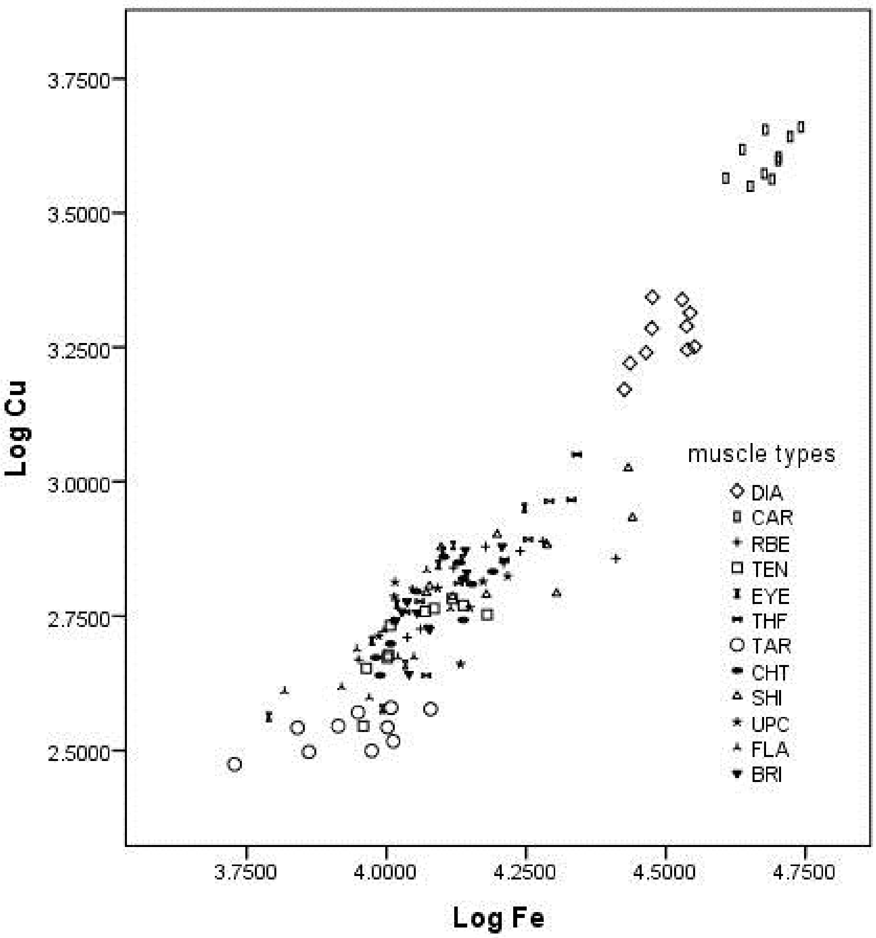

Table 1. Commercial veal cuts and muscles integrating each selected cut.

Commercial veal cuts	Muscles involved
Rib boneless entrecote (RBE)	*Longissimus thoracis* and *L. costarum*, *Spinalis dorsi*, *Multifidi dorsi*, *Complexus* and *internal/external intecostal*
Tenderloin (TEN)	*Psoas major*, *P. minor* and *Iliacus*
Eye round (EYE)	*Semitendinous* muscle
Thick flank (THF)	*Quadriceps femoralis*
Tail of rump (TAR)	*Tensior fasciae latae*
Chuck tender (CHT)	*Supraspinatus* muscle
Shin (SHI)	*Brachialis*, *Biceps*, *Coracobrachialis* and *Extensor carpo radialis*
Upper chunk (UPC)	*Rhomboideus*
Flank (FLA)	*Rectus abdominalis*
Brisket (BRI)	*Pectoralis profundus*

Table 2. Analytical quality program expressed as mean ± standard deviation used in the determination of essential and toxic trace elements.

	Detection limit (mg/Kg)	SRM 1577c	
		Certified levels (mg/Kg)	**Analysed levels (mg/Kg)**
As	0.0002	0.0196±0.0014	0.0119±0.0008
Cd	0.0002	0.0970±0.0014	0.1034±0.0052
Co	0.0002	0.300±0.018	0.320±0.006
Cr	0.0009	0.053±0.014	0.080±0.029
Cu	0.0276	275.2±4.6	291.1±10.6
Fe	0.3568	197.94±0.65	196.63±6.04
Hg	0.0006	-	0.147±0.020
Mn	0.0180	10.46±0.47	10.42±0.23
Mo	0.0057	3.30±0.13	3.35±0.35
Ni	0.0085	0.0445±0.0092	0.0724±0.0033
Pb	0.0017	0.0628±0.0001	0.0583±0.0054
Se	0.0273	2.031±0.045	1.849±0.052
Zn	0.2259	181.1±1.0	179.6±1.9

Table 3. Essential trace element (Co, Cr, Cu, Fe, Mn, Mo, Ni, Se and Zn) concentrations in muscles and liver tissue expressed as mean ± standard error of the mean. Essential trace element concentration means from muscular samples with a common letter following them do not differ significantly ($p < 0.05$).

Essential trace elements		Co (µg/kg wt.w.)	Cr (µg/kg wt.w.)	Cu (mg/kg wt.w.)	Fe (mg/kg wt.w.)	Mn (mg/kg wt.w.)	Mo (µg/kg wt.w.)	Ni (µg/kg wt.w.)	Se (mg/kg wt.w.)	Zn (mg/kg wt.w.)
Commercial Veal cuts	**RBE**	2.48±0.19 c	26.42±4.13 a	0.66±0.03 c	15.18±1.53 cde	0.85±0.01 cde	16.35±4.63 a	29.37±13.76 a	0.10±0.003 bc	48.35±4.96 ab
	TEN	1.92±0.14 c	46.77±12.54 a	0.52±0.03 cd	11.45±0.65 de	0.09±0.01 cd	42.99±13.32 a	28.75±9.24 a	0.11±0.005 bc	27.19±3.17 ef
	EYE	2.28±0.21 c	31.36±4.32 a	0.61±0.06 d	11.61±0.97 e	0.10±0.01 d	25.28±9.31 a	23.02±9.59 a	0.1±0.004 bc	36.99±3.02 def
	THF	2.52±0.30 c	26.16±4.23 a	0.75±0.07 c	16.07±1.38 cd	0.10±0.01 cd	16.42±4.97 a	10.04±1.20 a	0.09±0.004 c	41.86±2.76 abcd
	TAR	3.59±1.84 c	28.45±5.83 a	0.34±0.01 c	8.86±0.62 de	0.05±0.01 cd	20.31±8.30 a	15.95±4.54 a	0.10±0.004 bc	30.22±1.51 bcde
	CHT	2.12±0.11 c	40.86±10.70 a	0.60±0.03 c	12.41±0.66 cde	0.10±0.01 c	20.74±7.56 a	21.42±6.00 a	0.10±0.005 bc	42.69±1.78 abcd
	SHI	2.96±0.38 bc	44.45±12.82 a	0.73±0.05 c	17.45±1.88 c	0.12±0.01 c	18.69±5.53 a	11.79±0.10 a	0.10±0.004 bc	55.64±6.65 a
	UPC	2.16±0.28 c	45.86±10.50 a	0.59±0.02 cd	12.32±0.74 cde	0.10±0.01 cd	21.40±7.11 a	69.06±54.77 a	0.10±0.005 bc	44.34±1.88 abcd
	FLA	2.22±0.18 c	24.41±5.06 a	0.52±0.04 cd	10.88±1.10 de	0.07±0.01 cd	33.31±21.96 a	14.22±7.27 a	0.10±0.003 c	34.21±4.27 cdef
	BRI	2.41±0.16 c	43.62±10.45 a	0.60±0.03 c	12.19±0.59 cde	0.10±0.01 cde	21.67±7.54 a	19.26±2.50 a	0.11±0.004 bc	30.33±1.94 def
Internal muscles	**DIA**	3.99±0.11 b	41.88±8.52 a	1.87±0.07 b	31.63±1.08 b	0.23±0.01 b	36.13±14.94 a	18.45±2.45 a	0.12±0.01 b	47±1.34 abc
	CAR	10.77±0.48 a	25.50±6.95 a	4.02±0.12 a	48.14±1.39 a	0.41±0.02 a	48.302±5.44 a	13.56±3.98 a	0.22±0.01 a	19.97±0.55 f
	Liver	70.04±6.20	18.04±4.54	124.86±17.76	36.67±3.92	3.04±0.21	1243.36±42.85	16.63±5.33	0.53±0.08	41.04±1.24

RBE (rib boneless entrecote), TEN (tenderloin), EYE (eye round), THF (thick flank), TAR (tail of rump), CHT (chuck tender), SHI (shin), UPC (upper chuck), FLA (flank), BRI (brisket), DIA (diaphragm) and CAR (cardiac muscle).

Table 4. Toxic trace element (As, Cd, Hg and Pb) concentrations in different muscle types and liver tissue. Toxic trace element concentration means from muscular samples with a common letter following them do not differ significantly ($p < 0.05$).

	As		**Cd**		**Hg**		**Pb**	
Commercial veal cuts	**median**	**range**	**median**	**range**	**median**	**range**	**median**	**range**
RBE	1.61 ab	0.58-2.71	ND	ND-ND	0.866	ND-1.39	1.71	ND-5.12
TEN	1.76 b	1.36-3.37	ND	ND-1.23	0.766	ND-1.27	ND	ND-4.88
EYE	1.60 ab	0.93-3.13	ND	ND-0.169	0.491	ND-1.15	ND	ND-2.35
THF	1.54ab	1.02-2.37	ND	ND-0.810	0.557	0.415-1.04	ND	ND-2.21
TAR	1.53 ab	1.08-2.55	ND	ND-ND	0.863	ND-1.04	ND	ND-3.00
CHT	1.58 ab	0.96-3.39	ND	ND-0.465	0.572	0.409-1.11	ND	ND-5.44
SHI	1.49 ab	1.01-3.08	ND	ND-ND	0.791	ND-1.47	ND	ND-2.86
UPC	1.61 ab	1.35-3.14	ND	ND-0.779	0.671	ND-1.30	ND	ND-3.55
FLA	1.46 ab	0.88-2.30	ND	ND-7.64	0.359	ND-1.26	ND	ND-2.82
BRI	1.92 b	1.31-2.75	ND	ND-0.141	0.664	ND-1.27	1.78	ND-6.46
Internal muscles								
DIA	1.35 ab	0.83-2.60	ND	ND-ND	0.883	0.522-1.71	ND	ND-5.33
CAR	0.93 a	0.63-1.34	ND	ND-0.305	0.505	0.405-0.990	ND	ND-1.88
Liver	2.72	1.94-3.59	8.48	4.72-9			6.35	3.48-9.57

ND (Non-detectable concentrations) indicate residues below the limit of detection of the method.

RBE (rib boneless entrecote), TEN (tenderloin), EYE (eye round), THF (thick flank), TAR (tail of rump), CHT (chuck tender), SHI (shin), UPC (upper chuck), FLA (flank), BRI (brisket), DIA (diaphragm) and CAR (cardiac muscle).

Maximum admissible levels for meat products established by the European Commission are 0.05 mg Cd/kg wt.w. and 0.1 mg Pb/kg wt.w. (Commission Regulation (EC), No 1881/2006; Commission Regulation (EU), No 488/2014). No legal limits have been set for As and Hg in meat products.

Table 5. Spearman rank correlation coefficients and probability between pairs of essential and toxic trace element concentrations in all muscle samples analyzed in our study (n=120). Statistical significance of the correlations was indicated by * p<0.05, ** p<0.01 and *** p<0.001. Correlations were not calculated for Cd, Hg and Pb due to the high number of samples under the quantification limits of the method.

	Co	**Cr**	**Cu**	**Fe**	**Mn**	**Mo**	**Ni**	**Se**	**Zn**
Cr	0.11								
Cu	0.506***	0.006							
Fe	0.485***	0.01	0.851***						
Mn	0.254*	0.004	0.596***	0.640***					
Mo	0.311**	0.167	0.139	0.074	0.059				
Ni	0.132	0.381***	-0.058	-0.044	0.147	0.354***			
Se	-0.206*	0.133	-0.079	-0.166	-0.194	0.165	0.179		
Zn	0.291**	0.012	0.678***	0.704***	0.394***	0.004	-0.092	-0.043	
As	-0.131	0.254*	-0.014	-0.024	-0.03	0.156	0.314**	0.502***	-0.031