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Isabel Blanco-Penedo, Marta López Alonso, Marta Miranda, Joaquin Hernández, José Luis Benedito, Richard Frederick Shore

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I. Blanco-Penedo[a], M. López-Alonso[a*], M. Miranda[b], J. Hernández[a], J.L. Benedito[a], R.F. Shore[c]

[a]*Universidade de Santiago de Compostela, Departamento de Patoloxía Animal, Facultade de Veterinaria, 27002 Lugo, Spain.* [b]*Universidade de Santiago de Compostela, Ciencias Clínicas Veterinarias, Facultade de Veterinaria, 27002 Lugo, Spain.* [c]*Centre for Ecology & Hydrology, Lancaster Environment Centre, Library Avenue, Bailrigg, Lancaster LA1 4AP, UK.*

Abstract

We evaluated if differences in non-essential and essential trace element accumulation in beef-cattle reared under different systems (including organic, conventional and intensive management) were reflected in the meat derived from these animals. Diaphragm muscle from 166 calves from nine farms were analysed. Muscle cadmium concentrations were low (<10 µg/kg wet wt.) and muscle arsenic, mercury and lead concentrations were below limits of detection (<12, 2 and 3 µg/kg respectively) in most (77-97%) samples; there were no significant differences between farms. Essential trace element concentrations in muscle were generally within adequate physiological ranges and, although they varied significantly between farms, this was not apparently related to management practices. There were no significant correlations in element concentrations between muscle and liver or kidney (organ concentrations that better reflect exposure), except for cobalt

(positive association) and zinc (negative association). Non-essential and essential trace element concentrations in muscle in our study animals thus did not generally reflect differences in exposure. This is particularly relevant for animals reared in systems (such as organic farms) where cattle are exposed to somewhat higher levels of non-essential elements (probably due to soil ingestion when grazing) but also can suffer from mineral deficiencies.

Keywords: beef cattle; farming systems; non-essential elements; essential trace elements; muscle; nutrition.

*Corresponding author: M López-Alonso. E-mail: marta.lopez.alonso@usc.es.

Introduction

There have been various food scares over the past decade that has involved meat products. These include *Salmonella* in chicken, antibiotics in pork, *Escherichia coli* and banned growth promoters in beef, and the BSE crisis (Tarrant, 1998; Gambelli et al. 2003). These have contributed to a growing consumer awareness of food production methods that do not use chemicals and that are more in harmony with the natural environment. This, together with promotion of the importance of healthy food, has contributed to the recent rapid development of organic farming in the European Union (Vaarst and Hovi, 2004; von Borell and von Sorensen, 2004; Willer and Yussefi, 2006). Organic farming practices represent an alternative to the progressive intensification that has occurred in conventional animal production.

The quality of organic beef is affected by the production system used, particularly with respect to the grazing and exercise regimes which are integral components of any organic beef production system (Nielsen and Thamsborg, 2005). It is well documented that grazing cattle involuntarily ingest a certain amount of soil (up to 18%; Thornton and Abrahams, 1983) which can lead to a significant exposure to non-essential elements and other toxic compounds (such as pesticides, microbiological toxins, and medicinal products) that may be present in the soil (Falandysz et al., 1994; Sharpe and Livesey, 2005). This exposure scenario has also been described for other livestock species, such as organically-reared pigs (Linden et al. 2001) and hens (Kijlstra 2004). However, home-grown feed, and accompanying restrictions on mineral supplementation, can also lead to dietary deficiencies in organically farmed animals (Falandysz, 1993; Vaarst and Hovi,

2004). In particular, restrictions on feed supplements and prophylactic parasite control in organic beef production can lead to mineral deficiencies (Roderick and Hovi, 1999; MacNaeidhe, 2001) and result in poor body condition and production. Thus, there are potential conflicts between practices that are employed to ensure food safety and promote more "natural" livestock production and those used to enhance animal welfare (Vaarst and Hovi, 2004).

In terms of meat quality, different feeding systems have been studied to assess product quality in relation to post-mortem proteolysis, tenderness, meat flavour (Andersen et al. 2005) and chemical residues—mainly hormones and antibiotics (Smith et al. 1997). Although it has been well documented that farm practices can significantly affect assimilation by cattle of non-essential elements in key organs such as the liver and kidneys (López-Alonso et al. 2000), there is no information, as far as we are aware, on how can such practices affect non-essential and essential trace element concentrations in muscle, the main cattle product eaten by people.

We have recently examined how the assimilation of non-essential and essential trace element concentrations differs in the liver and kidneys of beef cattle in NW Spain between farms that vary in their production systems, particularly in the extent to which they graze cattle on pasture and provide dietary concentrates (Blanco-Penedo et al. 2008, 2009). Calves that were from farms which largely or exclusively grazed livestock on pasture and provided little or no mineral supplementation had the highest tissue concentrations of non-essential elements. We hypothesise that this is because pasture-grazed cattle ingest soil particles to

which non-essential elements adhere. There was also evidence of some essential trace element deficiency in cattle from farms that use low amounts of concentrates and do not provide mineral supplements. The objective of the current study was to evaluate if differences in non-essential and essential trace element accumulation by cattle that are related to farm production practices are reflected in meat destined for human consumption.

Material and methods

Farm selection

Farms were selected from the districts of Baralla (B), Montederramo (M) and Vilalba (V) in Galicia (NW Spain). In each district, a conventional (C), intensive (I) and organic (O) farm from the same neighbourhood were selected. The farms were similar in most respects other than their grazing and supplementary feeding regimes. Detailed information including farm size, feeding regime, other husbandry and management practices, and non-essential and essential trace element concentrations in soils and diet (forage and concentrate feed) have been summarised elsewhere (Blanco-Penedo et al. 2008, 2009).

Sample collection

A sample of diaphragm muscle (about 200g) was collected at the time of slaughter, which was when animals were between 7 and 10 months old. Samples were

packed in plastic bags, immediately placed on ice, transported to the laboratory, then stored at -18ºC until processing.

Sample analysis

Approximately 2 g sub-samples were digested in 5 ml of concentrated nitric acid (Suprapur grade, Merck) and 2 ml of 30% w/v hydrogen peroxide in a microwave digestion system (Milestone, Ethos Plus; Italy). Digested samples were transferred to polypropylene sample tubes and diluted to 25 ml with ultra-pure water.

Elements present at very low concentrations (arsenic (As), cadmium (Cd), chromium (Cr), cobalt (Co), nickel (Ni), mercury (Hg) and lead (Pb)) were determined by inductively coupled plasma mass spectrometry (ICP-MS; VGElemental PlasmaQuad SOption) whereas elements that occurred at higher concentrations (copper (Cu), iron (Fe), manganese (Mn), molybdenum (Mo), selenium (Se) and zinc (Zn)) were determined by inductively coupled plasma atomic emission spectrometry (ICP-OES; Perkin Elmer Optima 4300 DV). An analytical quality control programme was applied throughout the study. Blanks were run alongside samples and concentrations in samples were blank corrected as necessary. The limits of detection in the acid digest were calculated as three times the standard deviation of the reagent blanks (Table 1) and were based on the mean sample weight analysed.

Analytical Quality assurance

Analytical recoveries were determined from a Certified Reference Material (Pig kidney CRM 186, BCR Reference Materials, Belgium) that was analysed alongside

unknowns. There was generally good agreement between the measured and certified or indicative values (Table 1). The CRM was not certified for Co and Mo and analytical recoveries were determined for these elements using samples spiked at a concentration that gave absorbance values some 2-10 times greater than the normal levels in muscle. Mean recoveries were 89% and 96% respectively. The precision of the analytical method, calculated as the relative standard deviation (RSD) of Co and Mo concentrations in 10 digests of the same sample, were between 5.8 and 9.3 %.

Statistical analysis

All statistical analyses were done using the program SPSS for Windows (v.15.0). Non-detectable concentrations were assigned a value of half the detection limit when calculating mean element concentrations in muscle. Data were tested using a Kolmogorov-Smirnov test and were generally not normally distributed. They were therefore log-transformed before analysis, and average concentrations are therefore given as geometric means.

One-way Analysis of Variance followed by Tukey´s honest significant difference (HSD) *post-hoc* tests were used to test for differences in non-essential and essential trace element concentrations between farms. The significance of correlations between the levels of non-essential and essential trace elements in muscle and liver and kidney in each farm were calculated using Spearman rank correlation analysis. In all cases statistical significance was taken to be indicated by $p<0.05$.

Results and Discussion

Non-essential element concentrations in muscle from cattle from different farms are presented in Table 2. Overall, concentrations were low, and with the exception of Cd, most samples (96.6 % for As and Hg, 77.3% for Pb) had levels which were not detectable. The remainder of samples had concentrations close to the detection limit. Cd concentrations were all below 10 µg/kg wet weight. In general, non-essential element residues in meat in this study were similar to those described in previous studies in cattle in NW Spain (López-Alonso et al. 2000; 2004) and were within the range of those described in other countries (for review see López-Alonso et al. 2000). None of the samples analysed in the current study exceeded the maximum admissible levels for Cd and Pb (0.050 and 0.01 mg/kg fresh weight respectively) established by the European Commission (2001). The European Commission has not established statutory limits for As and Hg but Hg residues, when detected, were 1000-fold lower than those allowed in fish (European Commission 2001).

No significant differences were found between farms in muscle Cd concentrations (mean values ranged from 3.16 to 4.07 µg/kg wet weight) nor in the proportion of samples with detectable residues for the other non-essential elements (Table 2). There was no significant association between Cd concentrations in muscle and those in the liver or kidney (Table 3). This is broadly consistent with findings elsewhere that suggest that muscle concentrations of non-essential elements, except perhaps for As, are not closely related to the level of exposure, (Vreman et al. 1988).

Essential trace element concentrations in muscle from cattle from different farms are presented in Figure 1. In general, and with the exception of Se, essential trace element concentrations in cattle muscle were within the ranges described by Puls (1994) as adequate and were similar to those reported for cattle muscle in most other countries (for review see Jorhem et al. 1989; López-Alonso et al. 2000). In contrast, Se concentrations in cattle from all farms in the current study were above the normal range (0.070-0.150 mg/kg wet weight; Puls 1994) and were higher than concentrations reported in cattle elsewhere (Jorhem et al. 1989). Indeed, most animals in the present study had muscle Se concentrations within the high (0.250-0.500 mg/kg) or toxic (0.500-1.500 mg/kg) range. However, the ranges for essential element concentrations proposed by Puls (1994) are not comprehensive and do not take into account possible differences between different types of muscle. Comparison of results from separate studies superficially suggest that essential trace element concentrations in muscle vary 2-3 fold between cattle from different countries (Jorhem et al. 1989; López-Alonso et al. 2000) even when essential trace element concentrations in the liver and kidneys are comparable and within the adequate physiological range. However, differences in muscle element concentrations more likely reflected differences in the type of muscle analysed. For example, cattle diaphragm has been found to contain nearly twice the Cu and Se concentration of pectoral muscle (López-Alonso et al. 2000; López-Alonso *unpublished data*), perhaps reflecting greater metabolic activity in the diaphragm.

There were statistically significant differences between farms for most essential trace element concentrations in muscle (Figure 1). As with inter-farm differences in

liver and kidney concentrations (Blanco-Penedo et al. 2009), there was no evidence of any specific pattern of differences between regions or different types of farm. Cattle from the organic farm in Montederramo (MO) had the lowest mean Co, Cu, Ni and Se concentrations found in our study. This was the only farm where a high proportion of animals had hepatic Co and Se concentrations that were within the marginal or deficient range, and (together with the organic farm in Baralla (BO)), had calves with hepatic Cu concentrations that were below the range considered adequate (Blanco-Penedo et al. 2009). Calves from the MO farm also had significantly higher Zn muscle concentrations than cattle from other farms although, surprisingly, calves from the MO farm had the lowest mean Zn hepatic and renal concentrations that we recorded (Blanco-Penedo et al. 2009). The highest mean concentrations in muscle of most of the essential trace elements (Cu, Fe, Mn, Mo and Se) were in cattle from the intensive farm in Vilalba (VI), although it did not correspond with a higher intake of these elements in the diet or higher concentrations in the liver or kidney (Blanco-Penedo et al. 2009).

When analysing the relationship between essential trace element concentrations in muscle and those in the liver or kidney (data from Blanco-Penedo et al. 2009), the only significant positive association was for Co; muscle Co concentrations were positively associated with Co concentrations in both liver and kidney (Figure 2; Table 3). There was also a significant negative association between Zn concentrations in the muscle and those in the liver and kidney (Table 3). Taking into account that essential trace element concentrations in the liver, and to a lesser extent in the kidney, are the best indicators of the mineral status in calves (López-

Alonso et al. 2000), our results indicate that, at least under the conditions of our study, essential trace element concentration in muscle is generally not indicative of mineral status. This is presumably because muscle, unlike the liver and kidney, does not have essential trace element storage capacity. At adequate dietary intakes, mineral concentrations in muscle may be most closely related to protein synthesis and the predominant type of metabolism in the muscle (Schricker et al. 1982).

Although our results did suggest that muscle Co might correlate with Co status, this has not been found elsewhere. Van Ryssen et al. (1987) compared tissue Co concentrations in cattle given little or no dietary Co supplementation (Co requirement in cattle is 0.1 mg/kg DM; NRC 2001) with those in cattle given large Co supplements (10 and 40 mg/kg DM). The authors found that although liver and pancreatic Co concentrations significantly increased with Co supplementation (increase from 7.60 to 11.15 mg/kg dry weight in liver, and from 7.88 to 10.69 mg/kg dry weight in the pancreas), there was no concomitant increase in muscle Co (8.36 vs 8.56 mg/kg dry weight). It is possible that the low dietary levels of Co in cattle in our study were below those necessary to maximise metabolic activity (although normal or adequate Co concentrations have not been established for muscle; Puls 1994) and so Co concentrations in the muscle of cattle from farms where Co nutrition was better may have significantly increased alongside concentrations in the liver and kidney.

The negative association that we found between Zn concentrations in the muscle and those in the liver and kidney (Figure 3) was also surprising. In a previous study

in cattle with adequate Zn status in NW Spain (López Alonso 1999), Zn concentrations in the liver and kidney were strongly and positively correlated (as in this study; R_s=0.801, $p<0.01$) but there was no significant association between muscle Zn and either liver or kidney Zn. In cattle, Zn tissue concentrations are efficiently regulated by homeostatic mechanisms and, once optimal physiological concentrations (30 mg/kg DM; NRC 2001) are reached, Zn supplementation has no significant effect on Zn muscle levels (Kessler et al. 2003). Differences in tissue Zn concentrations between cattle receiving adequate Zn dietary concentrations could be due to factors such as age, sex, or production class (milk or beef cattle) (Puschner et al. 2004). It is also possible that other dietary components may influence the distribution of zinc in the body. For example other elements such as Cu and Cd have similar chemical and physical properties to Zn and compete for metabolic binding sites in metallothioneins (López-Alonso et al. 2002). It is possible that the negative association between Zn concentrations in the muscle and liver and kidney in the current study may be due, at least in part, to some of these confounding factors.

Conclusions

Our results indicate that differences in non-essential and essential trace element concentrations in the liver and kidney of cattle from different production systems on farms in Galicia are not reflected in the non-essential and essential trace element content in the meat. This is especially relevant for animals reared in systems (such

as organic farms) where weaned calves are exclusively reared by being grazed on pasture and, as a result, accumulate elevated hepatic and renal non-essential element levels (probably due to soil ingestion) but also can suffer from mineral deficiencies.

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Table 1. Results of the analytical quality control programme used in the determination of non-essential and essential trace elements

Element	Blank (n=16) Mean ± SD (µg/l)	Detection limit (µg/g)	Certified Reference Material	
			certified levels mean ± SD (mg/kg)	analysed levels mean ± SD (mg/kg)
As	0.98 ± 0.3	0.012	0.063 ± 0.009	0.069 ± 0.006
Co	0.04 ± 0.2	0.076	---	0.151 ± 0.054
Cr	0.5 ± 0.26	0.010	(0.058-0.142)	0.198 ± 0.043
Cd	0.10 ± 0.03	0.001	2.710 ± 0.150	2.711 ± 0.122
Cu	3 ± 1.9	0.072	31.9±0.4	29.1 ± 1.48
Fe	29.5 ± 1.66	0.063	299±10	283 ± 15.8
Hg	0.15 ± 0.05	0.002	1.970 ± 0.040	1.852 ± 0.111
Mn	0.69 ± 1.00	0.038	8.5±0.3	7.85 ± 0.51
Mo	0.58 ± 0.36	0.014	---	3.39 ± 0.29
Ni	0.95 ± 0.05	0.035	(0.420)	0.544 ±0.256
Pb	0.235 ± 0.09	0.003	0.306 ± 0.011	0.318 ± 0.041
Se	16.6 ± 8.33	0.317	10.3±0.5	11.9 ± 1.07
Zn	22.2 ± 2.50	0.095	128±3	128 ± 6.73

numbers in parentheses are indicative values

Table 2. Non-essential (As, Cd, Hg and Pb) element concentrations in muscle (µg/kg wet weight) in cattle in our study. Abbreviations for farms are as follows B: Baralla, M: Montederramo, V: Vilalba, C: Conventional, I: Intensive, O: Organic

Farm		As	Cd	Hg	Pb
BC	N (<ld)	13 (13)	13	13 (13)	13 (11)
	Geometric mean	ND	3.73	ND	1.81
	Range	ND-ND	2.10-6.13	ND-ND	ND-15.6
BI	N (<ld)	14 (13)	14	14 (14)	14 (12)
	Geometric mean	6.83	3.48	ND	1.75
	Range	ND-28.1	1.82-5.62	ND-ND	ND-11.7
BO	N (<ld)	14 (14)	14	14 (13)	14 (11)
	Geometric mean	ND	3.62	1.06	2.15
	Range	ND-ND	2.28-6.60	ND-5.56	ND-18.9
MC	N (<ld)	14 (14)	14	14 (13)	14 (11)
	Geometric mean	ND	3.16	1.03	2.13
	Range	ND-ND	1.87-4.86	ND-3.30	ND-18.0
MI	N (<ld)	13 (11)	13	13 (13)	13 (9)
	Geometric mean	6.95	3.57	ND	2.42
	Range	ND-14.5	2.16-7.05	ND-ND	ND-5.83
MO	N (<ld)	8 (7)	8	8(8)	8 (7)
	Geometric mean	6.82	3.20	ND	1.93
	Range	ND-14.5	2.02-5.81	ND-ND	ND-10.5
VC	N (<ld)	14 (14)	14	14 (14)	14 (11)
	Geometric mean	ND	4.07	ND	2.16
	Range	ND-ND	2.76-7.21	ND-ND	ND-8.91
VI	N (<ld)	14 (14)	13	14 (13)	14 (10)
	Geometric mean	ND	3.59	1.07	2.25
	Range	ND-ND	1.72-5.67	ND-5.78	ND-15.7
VO	N (<ld)	15 (15)	15	15 (14)	15 (11)
	Geometric mean	ND	4.02	1.05	2.17
	Range	ND-ND	1.76-8.93	ND-5.22	ND-7.20

N is the number of samples analysed and numbers in parenthesis indicates the number of samples that were below the limit of detection (N<LoD); ND: non detected

Table 3. Rank correlations between mean non-essential and essential trace element concentrations in muscle and liver and kidney in each farm in cattle in our study. Results are expressed as Spearman rank correlations coefficient and probability (*p<0.05, * p<0.01, *** p<0.001). Correlations have been not calculated for As, Hg and Pb because most samples had not detectable concentrations

Element (N)	Muscle *vs.* liver	Muscle *vs.* kidney
Cd (118)	-0.247	-0.165
Co (119)	0.875 **	0.913 ***
Cr (119)	-0.089	-0.367
Cu (164)	0.120	0.594
Fe (166)	-0.074	-0.137
Ni (119)	0.224	0.535
Mn (165)	0.608	0.325
Mo (165)	0.133	-0.587
Se (166)	0.256	-0.104
Zn (166)	-0.837 **	-0.704 *

Figure captions

Figure 1. Bar chart showing essential trace element concentrations in muscle (expressed as geometric means and geometric standard error) in the farms in our study. Abbreviations for farms are as follows B: Baralla, M: Montederramo, V: Vilalba, C: Conventional, I: Intensive, O: Organic. Different letters denote statistically significant differences between farms at $p<0.05$.

Figure 2. Scatterplot showing the relationship between Co concentrations in the muscle and liver and kidney.

Figure 3. Scatterplot showing the relationship between Zn concentrations in the muscle and liver and kidney.

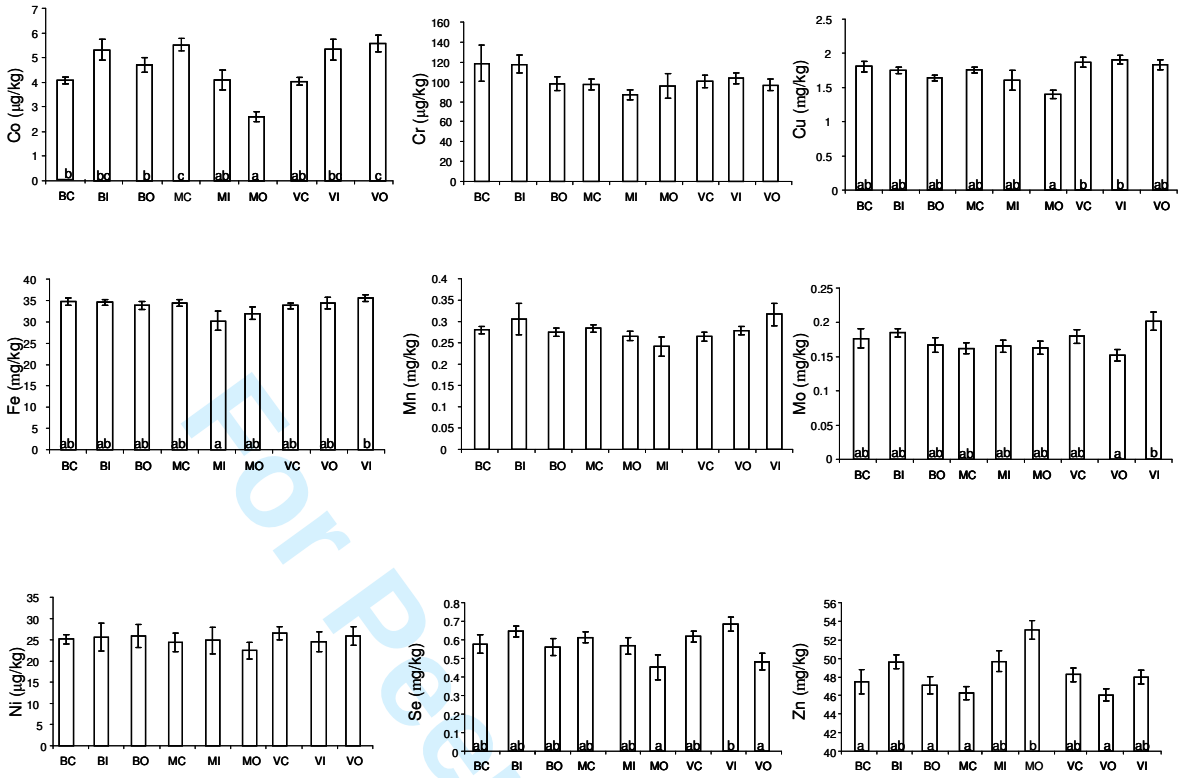
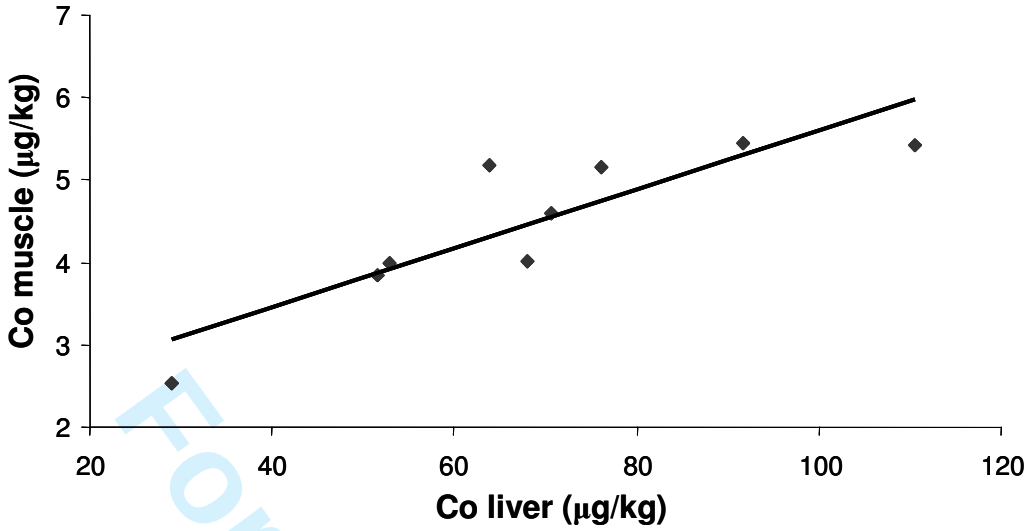


Figure 1.



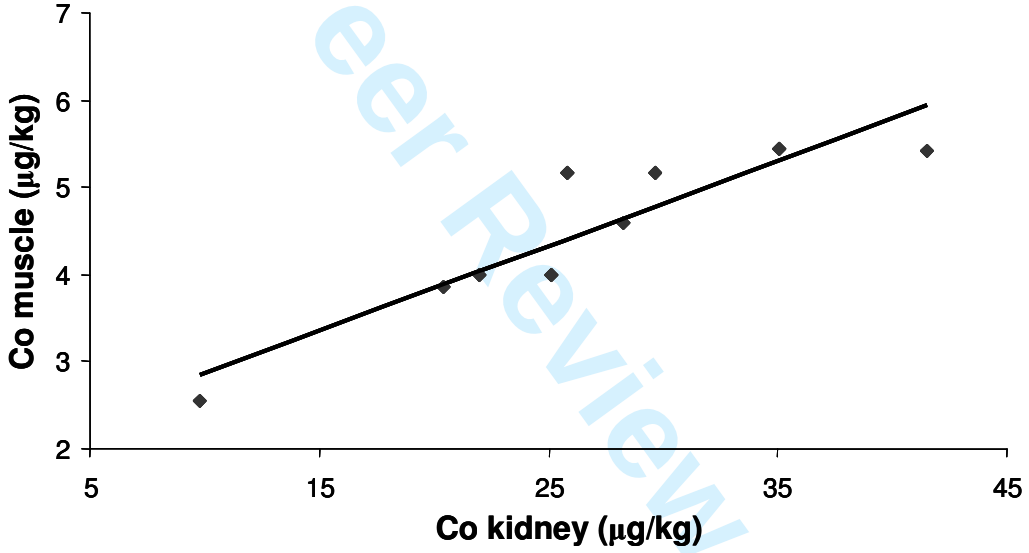


Figure 2.

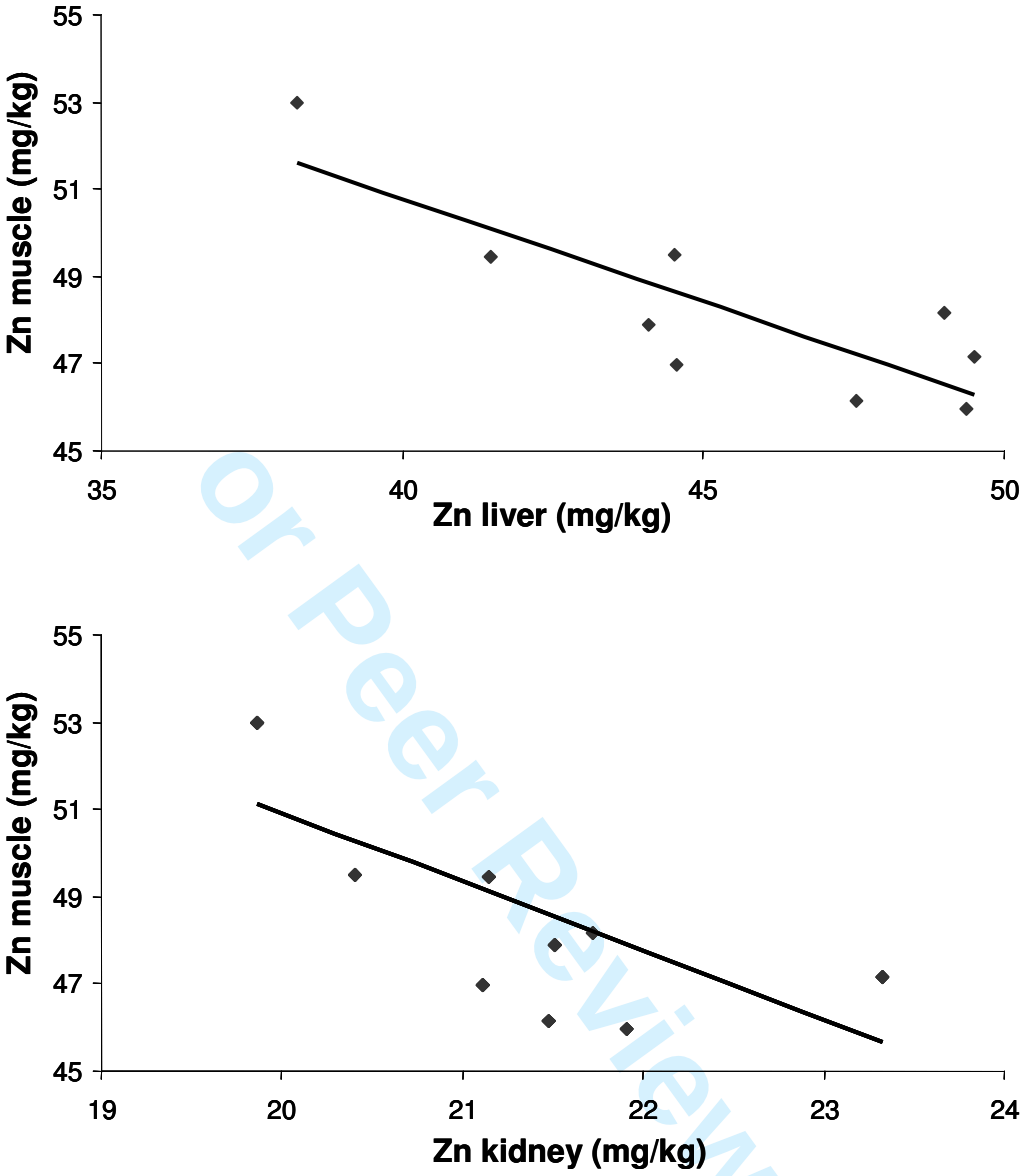


Figure 3.