# **Influence of Cu supplementation on toxic and essential trace element status in intensive reared beef cattle**

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## **Abstract**

The aim of this study is to evaluate if dietary Cu supplementation that leads to a hepatic Cu accumulation over the normal range has an influence on trace element status that could contribute to the pathogenesis of other mineral related disorders. Samples (liver, kidney, spleen, diaphragm and brain) of beef calves receiving typical commercial diets Cu supplemented and non-supplemented were tested for differences in non-essential and essential trace elements determined by ICP-MS. As (kidney and diaphragm), Hg (liver and kidney), and Pb (liver, kidney and spleen) were significantly lower, while Cd residues (liver and kidney) were significantly higher in the Cu supplemented group. Mn and Ni significantly decreased and Mo increased in the brain, and Se (diaphragm) decreased in the Cu supplemented group. These interactions are unknown, and possibly with more than two metals involved as suggested in the case of the ratio Se:Cu in the animals of this study. The possible role of Cu supplementation on the status of certain metals associated to neurological diseases (Mn–Ni) in the brain deserves further investigation. Finally new research on Cu–Se supplementation is necessary to better understand the risk of the animals to suffer from Se deficiency.

### Highlights

► Effect of dietary Cu on toxic-essential element accumulation in cattle tissues. ► As, Hg and Pb decreased while Cd increased in Cu supplemented animals. ► Ratio Se:Cu could be a tool to decrease toxic metal residues. ► Mn–Ni decreased and Mo increased in brain. ► Se decreased in diaphragm of Cu supplemented animals.

## **1. Introduction**

Traditionally, cattle were thought to be relatively tolerant to Cu accumulation and reports of Cu poisoning were, until recently, somewhat rare. In fact, Cu deficiency in cattle is a rather common disorder worldwide and cattle diets are regularly supplemented with high Cu concentrations. However, in the last years an increased number of episodes of Cu toxicity have been reported in the literature (Bidewell et al., 2000, VLA, 2001), even at liver Cu concentrations well below those classically regarded as toxic (Perrin et al., 1990, Gummow, 1996) and only sheep were demonstrated to be more susceptible than cattle to Cu toxicity (Underwood and Suttle, 2002). In most cases, cattle toxicity is associated with excessive Cu intake in the ration, as well as with changes in the type and bioavailability of dietary Cu supplements (Galey et al., 1991, Steffen et al., 1997, Laven et al., 2004). High Cu supplementation (up to 35 mg/kg dry matter (DM), the maximum level of Cu supplementation for cattle established by the European Union; Commission Regulation (EC) No. 1334/2003/EC), well above general physiological requirements (10 mg/kg; NRC, 2000) has been justified in most cases in view of the interference of Cu with other micronutrients, mainly molybdenum (Mo) and sulphur (S), but also iron (Fe) and zinc (Zn) (Kendall et al., 2001).

It has also been reported that dietary supplements leading to Cu accumulation in the liver at concentrations only slightly above normal (around 125 mg/kg wet weight (ww)) show negative effects on animal performance, in terms of reduced feed intake and average daily gain (Engle and Spears, 2000). The pathogenic mechanisms involved in this presumable subclinical chronic Cu toxicity during the chronic silent phase of hepatic Cu accumulation have not been elucidated. It is possibly that these negative effects of Cu could be attributed in part to reactive oxygen species (ROS) generation in cases of Cu overload. In rats hepatic toxicity due to Cu overload was attributed to ROS formation that are highly reactive and capable to damage all biological macromolecules (Britton, 1996, Luza and Speisky, 1996) and finally lead to apoptosis (Rana, 2008). In addition, it is possible that toxic effects of Cu could be related with the interaction with other micronutrients. For example, Cu interactions with other metals (mainly Mn, Zn and Fe) have been recently related with the pathogenesis of a great number of neurological diseases affecting both animals and humans, like for example Alzheimer’ disease (Maynard et al., 2005) and prion related diseases like spongiform encephalopathies in cattle (Tsenkova et al., 2004, Deloncle et al., 2006). High Cu dietary supplementation could also lead to a higher accumulation of toxic metals in the organism due to the capacity of this metal to increase metallothionein synthesis (Mercer, 1997); Rambeck et al. (1991) found that animals receiving for 3 months a diet supplemented with 200 mg/kg Cu showed cadmium (Cd) levels in the liver and kidney more than 2-fold higher than in control animals.

In a recent study of our research group in intensive beef cattle receiving a typical diet for commercial feedlots in Spain supplemented with 15 mg Cu sulphate/kg DM during all the productive cycle (García-Vaquero et al., 2011a) it was observed that a high proportion of animals (41%) had hepatic Cu concentrations associated with toxicity in cattle (>150 mg/kg ww: Perrin et al., 1990, Gummow, 1996), whereas in non supplemented animals blood Cu parameters and tissue Cu concentrations were within the adequate range. The aim of this study is to evaluate if dietary Cu supplementation that leads to a hepatic Cu accumulation over the normal range has an influence on non-essential trace element accumulation and essential trace element status that could contribute to the pathogenesis of other essential and non-essential element related disorders.

## **2. Materials and methods**

### **2.1. Experimental design and sample collection**

Tissue samples (liver, kidney, spleen, diaphragm and brain) of beef calves in which this study is based, were from a previous experiment to evaluate the need of Cu supplementation in intensively reared beef cattle by using Cu supplemented (with additional 15 mg CuSO4/kg DM, n = 10; Cu analyzed in the diet of 23 ± 8 and 26 ± 10 during growing and finishing periods, respectively) and non-supplemented beef cattle (no additional CuSO4, n = 10 Cu analyzed in the diet of 5 ± 1 and 8 ± 3 during growing and finishing periods). In the present study samples from 10 additional Cu supplemented animals were included (n = 30). These Cu levels were within the requirements established by NRC (2000). Detailed data of the experimental design and sample collection at slaughter are presented in García-Vaquero et al. (2011a).

### **2.2. Sample analysis**

For the liver, diaphragm, brain and spleen approximately 1 g of sub-samples were excised from semi-thawed tissues and acid digested. More details in García-Vaquero et al. (2011b).

The concentrations of non-essential (arsenic (As), cadmium (Cd), mercury (Hg), lead (Pb) and tin (Sn)) and essential (calcium (Ca), cobalt (Co), chromium (Cr), copper (Cu), iron (Fe), manganese (Mn), molibdenum (Mo), nickel (Ni), selenium (Se) and zinc (Zn)) trace elements were determined by inductively coupled plasma mass spectrometry (ICP-MS; VGElemental PlasmaQuad SOption).

An analytical quality control was applied throughout the study. Blank absorbance values were run alongside samples and these values were subtracted from the readings before the results were calculated. 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.

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

| **Element** |  |  | **Certified reference materials** | | | |
| --- | --- | --- | --- | --- | --- | --- |
|  |  |  | **CRM 186** | | **DORM-3** | |
|  | **Blank (n = 10)** | **Detection limit** | **Certified levels** | **Analysed levels** | **Certified levels** | **Analysed levels** |
|  | **Mean ± SD (μg/l)** | **(μg/g)** | **(mean ± SD; mg/kg)** | **(mean ± SD; mg/kg)** | **(mean ± SD; mg/kg)** | **(mean ± SD; mg/kg)** |
| As | 0.128 ± 0..006 | 0.0003 | 0.063 ± 0.009 | 0.0523 ± 0.0142 | 6.88 ± 0.300 | 6.07 ± 0.38 |
| Ca | 183 ± 18 | 0.801 | (295) | 337 ± 45 | – | – |
| Cd | −0.005 ± 0.002 | 0.0001 | 2.71 ± 0.15 | 3.25 ± 0.63 | 0.290 ± 0.020 | 0.276 ± 0.031 |
| Co | 0.025 ± 0.004 | 0.0002 | – | 0.109 ± 0.017 | – | 0.224 ± 0.033 |
| Cr | 0.916 ± 0.034 | 0.0014 | (0.058–0.142) | 0.067 ± 0.142 | 1.89 ± 0.17 | 1.20 ± 0.24 |
| Cu | 8.42 ± 0.71 | 0.0304 | 31.9 ± 0.4 | 39.9 ± 6.3 | 15.5 ± 0.63 | 15.5 ± 2.3 |
| Fe | −1.88 ± 3.06 | 0.136 | 299 ± 10 | 331 ± 36 | 347 ± 20 | 247 ± 31 |
| Hg | 0.024 ± 0.004 | 0.0002 | 1.97 ± 0.04 | 1.91 ± 0.21 | 0.382 ± 0.060 | 0.281 ± 0.037 |
| Mn | 0.229 ± 0.037 | 0.0016 | 8.5 ± 0.3 | 8.87 ± 1.51 | – | 2.06 ± 0.58 |
| Mo | 0.282 ± 0.107 | 0.0048 | – | 4.39 ± 0.79 | – | 0.644 ± 0.359 |
| Ni | 5.04 ± 0.06 | 0.0025 | (0.420) | 1.073 ± 0.940 | 1.28 ± 0.24 | 1.77 ± 0.35 |
| Pb | 0.225 ± 0.030 | 0.0013 | 0.306 ± 0.011 | 0.263 ± 0.040 | – | 0.270 ± 0.048 |
| Se | 0.186 ± 0.034 | 0.0015 | 10.3 ± 0.5 | 12.9 ± 1.8 | – | 3.81 ± 0.33 |
| Sn | −0.031 ± 0.003 | 0.0001 | – | 0.0068 ± 0.0405 | – | 0.0861 ± 0.0618 |
| Zn | 18.3 ± 21.1 | 0.9 | 128 ± 3 | 141 ± 13 | 51.3 ± 3.1 | 46.8 ± 5.6 |

∗Numbers in parentheses are indicative values.

Analytical recoveries were determined from certified reference materials (pig kidney CRM 186, BCR Reference Materials, Geel, Belgium; and fish protein DORM-3 National Research Council, Ottawa, Ontario, Canada) that were analysed alongside unknowns. There was good agreement between the measured and the certified or indicative values (Table 1). The two CRM used were not certified for Co and Mo and analytical recoveries were determined for these elements using samples spiked at a concentration that gave absorbance values 2–10 times greater than the normal levels. 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%.

### **2.3. Statistical analysis**

All statistical analyses were done using the program SPSS for Windows (v. 15.0). Non-detectable concentrations were assigned a value half the detection limit when calculating the mean element concentrations. Data were tested using Kolmogorov–Smirnov test and were generally not normally distributed, even after log-transformation. Therefore, Mann–Whitney test was used to test for differences in essential and non-essential trace element concentrations in liver, kidney, spleen, diaphragm and brain between Cu supplemented and un-supplemented animals.

Spearman rank correlation analyses were made between Cu concentration in the liver and essential and non-essential trace element residues in the rest of tissues to determine the influence of Cu supplementation in trace metal accumulation.

## **3. Results**

Non-essential trace element concentrations in liver, kidney, diaphragm, spleen and brain in beef cattle receiving a diet with (15 mg CuSO4/kg DM) and without Cu supplementation in this study are presented in Fig. 1. Overall, non-essential trace metals accumulated mainly in the kidney and in a lesser extend in the liver, whereas in the other tissues metal residues were very low with a high proportion of samples below the quantification limit (in %, Cd: 66.7 (spleen), 63.3 (diaphragm) and 56.7 (brain); Hg: 53.8 (liver); Pb 100 (diaphragm), 76.7 (brain); Sn: 100 (spleen), 86.7 (diaphragm), 83.3 (brain)) and did not differ statistically between themselves. Except for Sn, Cu supplementation significantly affected non-essential trace element accumulation in calves in our study. As residues were significantly lower in the kidney (53%) and diaphragm (41%), and tended (p < 0.10) to be lower in the liver (18%) and brain (6%) of Cu supplemented calves. Hg in the liver (52%) and kidney (19%), and Pb in the liver (65%), kidney (71%) and spleen (68%) were significantly lower in the Cu supplemented group. On the contrary, Cd residues were significantly higher in the liver (87%) and kidney (111%) of the Cu supplemented group.

**Fig. 1.** Box plot showing non-essential trace element concentration (μg/kg ww) in different tissues from non-supplemented (NS) and Cu supplemented animals (S). Abbreviations for tissue are as follows L: liver, K: kidney, D: diaphragm, B: brain. Different letters indicate statistically significant differences of trace element residues due to tissue type.Differences of metal accumulation due to Cu supplementation in the tissue were indicated as follows: T0.05 < p < 0.1, ∗p < 0.05, ∗∗p < 0.01, ∗∗∗p < 0001.

Essential trace element concentrations in the different tissues of cattle in this study are presented in Fig. 2. In general the highest trace element concentrations were found in the liver and/or kidney, except for Fe and Zn in which the highest levels were observed in the spleen and diaphragm, respectively. Cu supplementation hardly affected tissue essential trace element concentrations in calves in our study: Mn (13%) and Ni (54%) concentrations significantly decreased and Mo (83%) concentrations increased in the brain, and Se concentrations decreased in the diaphragm (15%) of the Cu supplemented group.

Fig. 2. Box plot showing essential trace element concentration (μg-mg/kg ww) in different tissues from non-supplemented (NS) and Cu supplemented animals (S). Abbreviations for tissue are as follows L: liver, K: kidney, D: diaphragm, B: brain. Different letters indicate statistically significant differences of trace element residues due to tissue. Differences of metal accumulation due to Cu supplementation in the tissue were indicated as follows: T0.05 < p < 0.1, ∗p < 0.05, ∗∗p < 0.01, ∗∗∗p < 0001.

Spearman rank correlations between Cu in the liver (as the most representative organ of Cu status in the animals) and essential and non-essential trace element concentration in the different tissues are presented in Table 2. Hepatic Cu concentrations were negatively related with As (Fig. 3) and Pb (Fig. 4) residues in most tissues. In relation to the essential trace elements, hepatic Cu accumulation was negatively related with Cr concentration in the brain and spleen (Fig. 5), Ni in the brain (Fig. 6) and Se in the diaphragm (Fig. 7).

Table 2. Spearman rank correlation coefficient and probability (∗p < 0.05, ∗∗p < 0.01, ∗∗∗p < 0001) between Cu liver concentrations and different essential and non-essential trace elements residues in liver, kidney, spleen, diaphragm and brain. Correlations for Sn in spleen and Pb in the diaphragm have been not calculated because most samples had undetectable concentrations.

|  | **Liver (n = 26)** | **Kidney (n = 26)** | **Spleen (n = 23)** | **Diaphragm (n = 26)** | **Brain (n = 26)** |
| --- | --- | --- | --- | --- | --- |
| As | −0.400∗ | −0.318 | −0.443∗ | −0.368 | −0.546∗∗ |
| Ca | 0.054 | −0.154 | −0.355 | 0.267 | −0.259 |
| Cd | 0.262 | 0.338 | −0.169 | −0.05 | −0.372 |
| Co | −0.297 | −0.223 | −0.034 | 0.281 | −0.314 |
| Cr | 0.206 | 0.255 | −0.581∗∗ | −0.355 | −0.491∗ |
| Fe | −0.236 | −0.05 | −0.244 | 0.241 | −0.015 |
| Hg | −0.141 | −0.277 | 0.027 | 0.114 | 0.059 |
| Mn | −0.085 | −0.219 | −0.183 | 0.188 | −0.166 |
| Mo | 0.059 | 0.054 | −0.303 | −0.234 | 0.221 |
| Ni | −0.101 | −0.077 | −0.257 | −0.116 | −0.446∗ |
| Pb | −0.519∗∗ | −0.685∗∗∗ | −0.653∗∗ | – | −0.203 |
| Se | −0.012 | −0.156 | −0.339 | −0.418∗ | −0.032 |
| Sn | 0.175 | 0.043 | – | 0.056 | 0.104 |
| Zn | 0.22 | −0.245 | 0.135 | 0.084 | 0.188 |

Fig. 3. Scatterplots showing the relationship between Cu concentrations in the liver and As in liver, spleen and brain. Grey: non-supplemented, Black: supplemented animals.

Fig. 4. Scatterplots showing the relationship between Cu concentrations in the liver and Pb in liver, kidney and spleen. Grey: non-supplemented, Black: supplemented animals.

Fig. 5. Scatterplots showing the relationship between Cu concentrations in the liver and Cr in spleen and brain. Grey: non-supplemented, Black: supplemented animals.

Fig. 6. Scatterplot showing the relationship between Cu concentrations in the liver and Ni in brain. Grey: non-supplemented, Black: supplemented animals.

Fig. 7. Scatterplot showing the relationship between Cu concentrations in the liver and Se in diaphragm. Grey: non-supplemented, Black: supplemented animals.

## **4. Discussion**

The results of our study have demonstrated that Cu supplementation in intensively reared beef cattle significantly affected toxic metal accumulation. Cd residues were significantly higher in animals receiving Cu supplementation. This could be related to a higher dietary Cd exposure in Cu supplemented animals, since mineral supplements generally contain significant concentrations of the main toxic metals (As, Cd and Pb); in fact mineral supplements are considered one of the main source of toxic metal exposure in farm animals from relatively unpolluted areas (Li et al., 2005, McBride, 2001). However, Cd contamination in the Cu mineral supplement does not explain the higher Cd levels observed in the supplemented animals in the current study since Cd concentration in the diet did not differ between non-supplemented (0044 ± 0004 mg Cd/kg DM, n = 10) and Cu supplemented animals (0042 ± 0005 mg Cd/kg DM, n = 10) and the other non-trace essential metal analyzed (As, Hg and Pb) decreased in the Cu supplemented animals.

On the contrary, Cd and Cu chemical interactions could explain, at least in part, the higher Cd residues found in Cu supplemented cattle in this study. Interactions between Cu and Cd have been widely reported in mammals at different levels of metal exposure and are a consequence of the shared ability of these metals to induce synthesis and compete for the binding sites of metallothionein (MT) (Bebe and Panemangalore, 1996, Coudray et al., 2006, Frank et al., 2000, Komarnicki, 2000; López-Alonso et al., 2002; Phillips et al., 2005, Reeves and Chaney, 2004, Taylor, 1996). In other farm species, intensively reared pigs supplemented with 200 mg/kg Cu for 3 months showed Cd levels in the liver and kidney more than 2-fold higher that in control non-supplemented animals (Rambeck et al., 1991). Even though no information is available on tissue Cd accumulation in cattle receiving Cu supplementation, numerous examples of naturally occurring interactions between Cu and Cd have been reported (Nriagu et al., 2009, Waegeneers et al., 2009) and cattle hypocupraemia has been frequently described in areas with Cd polluted soils (Koh and Judson, 1986, Miranda et al., 2005, Prankel et al., 2005, Spierenburg et al., 1988, Wentink et al., 1988).

Interactions between Cu and the other main toxic metals (As, Pb and Hg) are less known, and as far as we are aware, there is no information on the effect of Cu supplementation on toxic metal accumulation in cattle or other farm animals. Even though there are examples of interaction between Cu and these toxic metals in the scientific literature, these have been described in naturally exposed animals (Blanco-Penedo et al., 2006, López-Alonso et al., 2002, Miranda et al., 2005, Nriagu et al., 2009) or in laboratory mammals exposed to high metal concentrations (Agarwal et al., 2010, Dhawan et al., 1995, Rambeck et al., 1991) well above those found in normal diets.

There are some examples of Pb–Cu interactions in the literature. In experimental studies in mice, administration of a high Pb diet has been reported to lead to decreased Cu absorption and thus decreased Cu levels in the liver (Dhawan et al., 1995); whether Pb–Cu interaction is at the site of absorption of Cu in the gastrointestinal tract or at some other metabolic or transport site has not been determined. Conflicting results have been obtained regarding Cu–Pb interaction in cattle. Some studies have reported negative associations between Cu and Pb in the liver of animals exposed to low environmental Pb (Miranda, 1999, Spierenburg et al., 1988), while others have found no statistically significant association (López-Alonso et al., 2002) or a strong positive correlation between both elements in the liver (Blanco-Penedo et al., 2006, López-Alonso et al., 2004). Although the Cu–Pb interaction mechanisms are unknown, it has been suggested that the positive associations between Cu–Pb could be related to a common source of exposure of both metals by soil ingestion (Blanco-Penedo et al., 2006, López-Alonso et al., 2004).

Scarce information is available on interactions between Cu and As in cattle. A strong negative correlation was found between Cu and As concentrations in the liver of cattle with a very low Cu status (Nriagu et al., 2009). In previous studies in our region As concentrations in cattle were very low, with a large number of animals showing non-detectable As residues in the liver, kidney and diaphragm (Blanco-Penedo et al., 2006, Blanco-Penedo et al., 2010, López-Alonso et al., 2000a, López-Alonso et al., 2000b). In these studies animals were raised in farms with outdoor grazing throughout the year in areas with high Cu contents in soil and forage, both from a natural origin (serpentine soils) or due to the use of high Cu pig slurry as fertilizer (López-Alonso et al., 2000a; Miranda et al., 2009). On the light of the results of the present study, it could be possible that the low As accumulation in Galician cattle in these previous studies, even though As concentrations are relatively high in some soils in Galicia (López-Alonso et al., 2000a), could be related to the high level of Cu exposure.

As far as we are aware, there is a lack of studies showing the interaction between Cu and Hg in cattle. On the contrary, it has been well documented that Hg and Se interact biologically, and the coadministration of both reduces the toxicity of each other (Cuvin-Aralar and Furness, 1991), although the mechanism underlying this protective effect is still unsolved. In the cattle in the present study there was a significant decrease in Hg residues in the liver and kidney of the Cu supplemented animals, although no significant correlations were found between hepatic Cu and tissue Hg concentrations. Metal interactions can be very complex, with more than two metals involved, and their effects highly dependent of the relative concentration of metals in the organism (López-Alonso et al., 2004) and so, in this study the correlations between the ratio Se:Cu in the liver and As (liver, kidney, diaphragm and brain), Hg (kidney) and Pb (liver, kidney and spleen) (Fig. 8) are difficult to explain. When evaluating essential trace element concentrations in cattle in this study it was observed, that both Cu unsupplemented and supplemented animals showed an adequate mineral status, since essential trace elements concentrations in the liver and kidney (the tissues more common to evaluate the adequate mineral status) were within the ranges described as adequate (Puls, 1994, Underwood and Suttle, 2002). These results indicate, in a first instance, that the typical commercial diet for beef cattle in intensive systems covers the physiological requirements, and mineral supplementation routinely used in intensively fed cattle could not be justified to maintain nutritional requirements. Cu supplementation showed a significant influence on essential trace element tissue concentrations. Interestingly, the brain was the tissue that showed the highest differences between both groups of animals: Cu supplemented animals showed statistically significant lower Mn, Mo and Ni concentrations compared to the unsupplemented calves.

Fig. 8. Scatterplots showing the relationship between Se:Cu ratio in the liver and As (liver, kidney, diaphragm and brain), Hg (kidney) and Pb (liver, kidney and spleen). rs: Spearman rank correlation coefficient, p: probability. Grey: non-supplemented, Black: supplemented animals.

Cu and Mn concentrations in the brain have acquired great relevance in the later years due to the role of Cu in the biology of prions related with transmissible spongiform encephalopathies (TSE) like bovine spongiform encephalopathy (BSE). The binding of Cu ions by the prionic protein stabilize the protein that may play a role in the antioxidant defense via its Cu-dependent superoxide dismutase like activity (Brown et al., 1997, Brown et al., 1999, Wong et al., 2000). Imbalances in Cu and Mn that allow for Mn ions to replace Cu on the prionic protein result in changes in the biochemical characteristics of the molecule (Deloncle et al., 2006, Tsenkova et al., 2004), large decreases in brain Cu and increases in Mn have been associated with TSE (Thackray et al., 2002, Wong et al., 2001). In Cu deficient cattle, the Cu status affected bovine brain Cu concentrations but had not detectable effects on brain Mn concentrations or prion protein characteristics (Legleiter et al., 2007). In the present study Cu supplementation in beef cattle lead to an increase of Cu concentrations in the brain together with a significant decrease in Mn concentrations; this result could attribute to Cu supplementation a beneficial or protective effect on the prevention of the BSE, especially in cases where the balance of Cu and Mn in the diet is not appropriate.

Evidences of a competitive interaction between Cu and Ni were previously reported in rats: Ni and Cu have similar binding components in serum and the same specific transport site in albumin (Nielsen, 1987) and in Cu deficient rats, clinical signs of Cu deficiency (like elevated plasma cholesterol and depressed haemoglobin) were exacerbated by Ni supplementation (Nielsen et al., 1979, Nielsen and Zimmerman, 1981). In ruminants Cu–Ni interaction is not clear, and Cu concentrations in the liver, kidney, spleen, lung, heart and muscle were not affected in calves supplemented with 5 mg Ni/kg in the diet (Spears et al., 1986). The results of the present study concerning the diminish of Ni in the brain of Cu supplemented animals could be relevant since the effects of Ni in the nervous system are still scarce (Senatori et al., 2009) and several studies in the recent years are focused on the Cu and Ni binding to histidine containing peptides involved in neurodegenerative disorders such as Alzheimer’s and prion disease (Zoroddu et al., 2004, Zoroddu et al., 2008, Zoroddu et al., 2009).

As far we are concerned, no information is available in the literature about interactions between Cu and Cr in the brain or other tissues that allow us to explain the mechanisms of the Cu–Cr negative interaction in our study. Anyway, the negative correlation between Cu and Cr concentrations in the spleen could have a clinical implication in animals receiving high dietary Cu supplementation, since recent studies in cattle have attributed to Cr a major role in both humoral (Almeida and Barajas, 2001, Almeida and Barajas, 2002, Burton et al., 1994,) and cell-mediated immunity (Burton et al., 1993) having the spleen a mayor role in lymphocytes B and plasma cells production (Fry and McGavin, 2007).

Finally, a significant negative interaction between Cu and Se in the diaphragm was observed in calves in our study, animals receiving Cu supplementation showing significantly lower Se concentrations in this muscle. It has been demonstrated that high Cu intakes reduce intestinal absorption of Se in ruminants because highly insoluble Cu selenide compounds are formed in the rumen (Koenig et al., 1991). Experimental studies in ruminants have also demonstrated that after injection of Se, Cu concentration in the liver increased in animals receiving both normal (Hussein et al., 1985) or Cu deficient diets (Thomson and Lawson, 1970) and that this could be related to metallothionein synthesis in the liver after Se treatment (Chimielnicka et al., 1983). Previous studies in cattle in NW Spain have found significant positive correlations between Cu and Se in the liver (López-Alonso et al., 2004) or in the kidney (Blanco-Penedo et al., 2006) but not negative interactions in the muscle, as in this study. These differences in Cu–Se interactions between studies could be related, as indicated above, with the relative concentrations or ratios of both elements in the organism. The effect Cu supplementation on Se concentrations in the muscle could have a clinical relevance since a lack of Se was associated with several muscular diseases affecting both cardiac and skeletal muscles in cattle, like the white muscle disease, characterized by extensive calcification of cardiac and skeletal fibers; emergence of these diseases is strongly influenced by diet and Se supplementation is important to avoid these disorders (Rederstorff et al., 2006).

## **5. Conclusions**

Under the nutritional conditions of cattle in this study, which are the typical in intensive systems in many European countries, Cu supplementation was associated with a higher Cd accumulation. On the contrary, Cu supplementation lead to a lower As, Pb and Hg accumulation in most tissues, and although the mechanisms of these interactions are unknown, and possibly with more than two metals involved as suggested in the case of the ratio Se:Cu in the animals of this study. The possible role of Cu supplementation on the status of certain metals associated to neurological diseases (mainly Mn and Ni) in the brain deserves further investigation. Finally, due to the negative effect of Cu supplementation on Se muscular status in calves in this study, new research on Cu–Se supplementation is necessary to better understand the risk of the animals to suffer from Se deficiency.

## **Conflict of Interest**

The authors declare that there are no conflicts of interest

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