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Forage enrichment with copper and zinc in beef grazing systems in Argentina

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ABSTRACT

The presence of trace elements in water and soils, their absorption by plants, and their transfer to the food chain, is a phenomenon that has been studied in different regions around the world. In Argentina, few studies exist regarding trace element content in soils, and the studies on forages are furthermore scarce. The interest to quantify the levels of trace elements in cattle forage lies in the intrinsic connection between the quality of forage and the nutritional (i.e. Cu and Zn) aspects of cattle health and products for human consumption. Feed lots use feed, which incorporates core minerals whose basic components are Fe, Se, I, Mn, Co, Cu, and Zn. The excess of these trace elements are eliminated through the feces into the environment, therefore, the use of manure from these systems, as a source of organic fertilizer, could reduce environmental contamination, and supplement soils, in areas which are naturally deficient in these micronutrients, for forage growth intended for livestock consumption. Soils from different areas in Argentina were characterized, mainly for their Cu and Zn content. Soil–plant transfer of these trace elements to natural forage was analyzed for agricultural soils with higher content of micronutrients. On the other hand, pot experiments were performed for the enrichment of the forage species, *Lolium perenne* (ryegrass), with Cu and Zn through fertilization with bovine feces spiked with known concentrations of these trace elements. Soils from copper-deficient areas used for livestock breeding were used in these experiments. Differences in Cu and Zn concentrations were observed between the fertilized and non-fertilized reactors, while there were no differences found in the Cu and Zn concentrations considering the method of fertilization (homogenization or superficial). These results show that manure from feed lots may be used for forage enrichment with micronutrients.
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1. Introduction

Microelements are defined as elements that are present at trace concentrations in most soils, plants and organisms. They can enter in agroecosystem through both natural and anthropogenic processes (He et al., 2005). In soils destined to agricultural activities, microelements load could be attributed to atmospheric deposition, discharges, livestock manure, agrochemicals and organic fertilizers (Nicholson et al., 1999).

The presence of trace elements in water and soils, their absorption by plants, and their transfer to the food chain are a phenomenon that is being studied in different regions around the world. In Argentina, few studies exist regarding trace element content in soils (Heredia and Fernández Cirelli, 2009; Lavado, 2006; Lavado et al., 1998, 2004), and the studies on forages are furthermore scarce. The interest to quantify the levels of trace elements in cattle forage lies in the importance of micronutrients for animal health and the intrinsic connection between the quality of forage and the nutritional and toxicological aspects of livestock products for human consumption (Miller et al., 1991).

The most naturally occurring mineral deficiencies in ruminants are associated with specific regions and are directly related to soil characteristics (Suttle et al., 1980). Copper (Cu) deficiency is of common occurrence in bovine production. In Argentina, hypocuprosis has a clear geographical relationship, resulting from soil characteristics and a particular topography, which directly influence forage growth and Cu availability (Ramírez et al., 1998).

Zinc (Zn) deficiency is the most important problem in alkaline-calcareous soils (Gupta et al., 2008). Factors that induce Zn deficiency include soil pH, soil calcareousness, low soil organic matter, sandy soil texture, eroded soils, and accentuated Zn mining by high yielding varieties (Rashid and Ryan, 2004). Zinc deficiencies mainly occur on sandy soils, on soils with a pH > 6.0, and hence, continuous fertilization without supplementation of Zn may induce Zn deficiencies. It is mobile at slightly acidic conditions and is immobilized in alkaline soils. Antagonism of zinc in soil, e.g., with phosphorus (P), calcium (Ca), Fe, Cu, and nickel (Ni) has been reported (Deckers and Steinnes, 2004; Gupta et al., 2008).

The Pampean plain of Argentina has different kinds of soils with different aptitude for agriculture and different micronutrient content. In areas where important cattle grazing systems are established,

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deficiencies in copper and zinc have been reported (Lavado, 2006; Lavado, et al., 2004). On the other hand, feed lots system are in expansion in Argentina in the last 20 years, due to the continuous increase in the surface used for soya bean crops (INDEC, 2002; SENASA, 2010). These confined cattle production systems use feed which incorporates core minerals whose basic components are Fe, Se, I, Mn, Co, Cu, and Zn. Trace element content in manure of intensive animal production systems is related to feed mineral content and the animal conversion efficiency (Nicholson et al., 1999). Increases in metal concentration in animal feed have often resulted in corresponding increases in their concentration in manure by-products (Nahm, 2002).

With increasing use of trace elements as nutritional supplement in the form of feed additive in intensive animal production systems, manure application appears as an important source of certain metals input in soils (Bolan et al., 2004). Use of manure and composted biosolids has been reported to increase total amounts of Cu, Zn, Pb, Cd, Fe and Mn in the soils (Mc Bride, 2004). Metals accumulate in soils since they are not biodegradable with potential environmental impact not only in soils but in water and also the possibility of becoming phytotoxic (Yassen, et al., 2007).

In previous works, trace metals in manure, soils and sediments from the drainage canalizations in feed lots systems in Argentina were analyzed. Metal-enriched feces have resulted in elevated concentrations of metals in soils, mainly Cu and Zn (Moscuzza and Fernández Cirelli, 2009). Metal accumulation appears to be directly proportional to the age of emplacement of the establishment.

The reuse of excrement from these systems, as a source of organic fertilizer, could reduce environmental contamination, and supplement soils, in areas which are naturally deficient in these micronutrients, for forage growth intended for livestock consumption.

In the present study soils from different areas in Argentina were analyzed for their Cu and Zn content. In areas with higher levels of these trace metals, forage was also analyzed. On the other hand, pot experiments were performed using poorer soils to test the efficiency of forage enrichment through fertilization with composted manure from feed lots systems.

2. Materials and methods

2.1. Sampling

2.1.1. Soil

Soils samples (n = 17) were collected in agricultural areas of different regions of the chaco pampean plain in Argentina. Samples were obtained using an auger at 0–30 cm depth, all samples from a representative area were mixed in order to obtain a homogeneous sample (1 kg), and preserved at 4 °C in a double plastic bag.

2.1.2. Forage

Forage samples (alfalfa—*Medicago sativa*) were collected in correspondence with the soil samples from a representative area, were mixed in order to obtain a homogeneous sample (1 kg), and preserved at 4 °C in a double plastic bag.

2.2. Samples preparation and analysis

2.2.1. Soil

Soil samples were air dried, passed to a 2-mm sieve and analyzed for extractable phosphorus (Bray–Kurtz 1 method), pH (1:2.5 soil–water relationship), organic carbon (OC) (Walkley–Black method), electrical conductivity (EC), and total nitrogen (TKN) (Microkjeldahl method) as described in Sparks (1996) and USDA (1996). For determination of trace elements content, 0.1 g of soil was subjected to acid digestion with 10 ml of 10 N HNO_3 (c) and 10 ml of 15 N HSO_4 (c). After the complete oxidation of organic matter, and produced fumes of sulfur trioxide, the samples were cooled and diluted to 50 ml with deionized water.

2.2.2. Forage

Samples were washed with distilled and deionized water and air dried. The disintegration of the vegetable samples was performed by microwave digestion in closed cup (Alvarado, 1996).

2.3. Trace elements analysis

Copper and zinc content in soil and forage was determined by inductively coupled plasma, optical emission spectroscopy (ICP-OES, Perkin Elmer, Optima 2000) following standardized methods (APHA, 1998). Determinations were performed in triplicate being the relative error < 1.0% for all of them.

2.4. Pot culture experiments

The experimental plants were ryegrass (*Lolium perenne*). Soil samples (soil nº 10, Table 1) were air dried, passed through a 2-mm sieve and placed in plastic-lined pots (1 kg/pot).

Feces were collected from bovines without supplementation, air dried and passed through a 2-mm sieve. The Cu and Zn solutions were made with increasing concentrations of copper and zinc sulfate. Three solutions were made: (1) (SN1) containing 7.5 mg/L of Cu and 60 mg/L of Zn, (2) (SN2) containing 25 mg/L of Cu and 200 mg/L of Zn, and (3) (SN3) containing 50 mg/L of Cu and 400 mg/L of Zn. Each solution was mixed by stirring with 1 kg of feces. These mixtures of Cu and Zn were incubated for 15 days at room temperature. The fertilization rate with excreta in each reactor was based on the N concentration. The total Kjeldahl nitrogen in composted manure was 2.0%. A fertilization rate of 250 kg total N/ha was considered.

For each concentration eight reactors were used. In four of them, fertilization was performed by homogenization through a mixed fraction of the soil with manure; the other four were fertilized at the surface only. Eight reactors were used as controls, four with excreta and without addition of Cu and Zn, and 4 reactors without fertilization at all.

To determine Cu and Zn contents, forage samples were digested by microwave and analyzed by ICP-OES techniques (APHA, 1998). In all cases, tests were performed in triplicate with a relative error less than 1%.

Statistical analyzed was based in repeated-measures analysis of variance (ANOVA) among different treatment (without fertilization, fertilization with homogenization and superficial fertilization) and multiple comparisons test Tuckey's honestly significant differences (HSD) (Zar, 1999). $P<0.05$ was considered statistically significant.

The compound symmetry and sphericity assumption of repeated-measures analysis were tested a priori by the Box's M statistic test and Mauchley's sphericity test respectively ($P<0.05$). The software used in statistical analysis was STASTICA 5.1 (STATSOFT®, 1999).

2.5. Reagents

All reagents were of analytical grade. Working solutions were prepared by appropriate serial dilution of commercially available multielements stock standard solutions (Perkin Elmer. Atomic Spectroscopy Standard Nº 9300281) using ultrapure water provided by a Milli-Q water purification System (Millipore, Bedford, MA, USA).

Certified reference materials for verification of the calibration procedure and validation of the analytical method were used for each type of studied matrices. In the case of soils, WQB CRM-3, and HR-1 were used (NWRI) and for forage, NIST-1570a, from the National Institute of Standards and Methods (NIST), was used.

3. Results and discussion

Physicochemical characterization and Cu and Zn content of soil from different areas of the Chaco pampean plain are shown in Table 1.

Table 1
Physicochemical characterization and Cu and Zn content of soil from different areas of the Chaco pampean plain.

Soil	Lat (S)	Long (W)	Clay (%)	Silt (%)	Sand (%)	Soil texture	pH	EC (ds/cm)	ExtP	OC (%)	TKN	Cu (mg/kg)	Zn (mg/kg)
1	32° 37′	62° 34′	22.5	57.5	20	Silt loam	7	0.9	32.8	1.4	0.20	76.1	126.4
2	32° 42′	62° 33′	40	50	10	Clay loam	7.4	1.5	24.8	1.3	0.11	93.1	127.6
3	32° 38′	62° 35′	37.5	47.5	15	Clay loam	6.5	0.3	84.9	2.5	0.32	63	112.8
4	32° 37′	62° 40′	35	57.5	7.5	Clay loam	6.5	0.3	30.1	2.2	0.24	31.4	77.1
5	32° 30′	62° 47′	27.5	57.5	7.5	Clay loam	6.5	0.3	28.7	2.2	0.27	85.6	129.4
6	32° 39′	62° 45′	27.5	52.5	20	Silt loam	6.9	0.4	33.6	1.6	0.22	34.6	60.8
7	32° 34′	62° 36′	17.5	32.5	50	Loam	6.8	0.3	51.2	1.3	0.23	31.4	61
8	32° 34′	62° 34′	27.5	57.5	15	Silt loam	6.8	0.5	38.6	2.0	0.26	52.8	93.7
9	32° 28′	62° 40′	15	62.5	22.5	Silt loam	7.1	0.6	11.4	1.3	0.24	74.3	121.7
10	35° 21′	59° 30′	21	40	39	Loam	6.8	0.5	9.6	0.8	1.43	7.1	31.8
11	35° 16'	57° 46'	26.8	58	15.2	Silt loam	6.8	0.8	12.8	2.4	0.23	20.4	37.5
12	36° 18'	57° 22'	29	34	37	Clay loam	7.7	2.5	14	1.8	0.22	7.5	33.2
13	34° 42'	59° 28'	22.5	62.5	15	Silt loam	6.6	0.3	10.5	2.3	0.25	15.1	47.1
14	33° 55'	59° 24'	25	55	20	Silt loam	6.0	0.5	16.9	1.9	0.21	19.4	54.2
15	33° 50'	59° 27'	25	50	25	Silt loam	6.1	0.3	43.2	2.9	0.25	19.9	58
16	35° 51'	58° 54'	18	49	33	Loam	6.9	0.6	7.3	2.0	0.22	10.8	31.4
17	33° 22'	65° 53'	10	45	45	Loam	7.45	0.48	4.9	0.8	0.05	12.8	46.1

EC: electric conductivity, Ext P: extractable P, Lat: latitude, Long: longitude, OC: organic carbon, TKN: Total nitrogen.

Soils 1–9 are from the southeast of Córdoba province and are typical agricultural soils from the pampean plain in Argentina with higher nutrients and micronutrients content. In this area, Cu concentration ranged from 31.4 to 93.1 mg/kg, whereas Zn concentration ranged from 60.8 to 129.4 mg/kg. These results are in accordance with the reported values in other agriculture regions (Kabata-Pendias and Pendias, 2001; Lavado et al., 2004).

Soils 10–16 are from different regions of the province of Buenos Aires. Soil 15 is from northeast Buenos Aires, characterized for its aptitude for crops. This soil exhibits similar physicochemical characteristics and nutrient content to those from Southeast of Córdoba. The other soil samples belong to areas of livestock production, with Cu contents raging from 7.5 to 20.4 mg/kg and Zn from 31.4 to 58 mg/kg. Soil 17 is from the center of San Luis province in the semiarid region of Argentina (mean annual rainfall 500 mm) characterized by sandy soils with poor organic matter content. Cu and Zn also showed low values when compared with those from the humid agriculture area (mean annual rainfall from 800 to 1000 mm).

Typical forage in Cordoba province, whose soils showed higher Cu and Zn concentration is alfalfa (*Medicago sativa*). This crop is highly sensitive to Cu deficiency, while Zn deficiency is often found in temperate climates (Gupta et al., 2008).

No studies have been reported in our country of the micronutrient content of this species. In Table 2, Cu and Zn contents from plants sampled in the southeast of Cordoba province are shown (number of alfalfa sample is in correspondence with the number of soil sample).

Cu levels were between 8.2 and 15.2 μg/g, considered an appropriate value for this crop (Gupta et al., 2008). Values of 9.9 μg/g have been reported. Zn levels were between 20.2 and 56.8 μg/g, which comprise in the reported values in the literature (Gupta and Karla, 2006; Gupta et al., 2008).

In this agricultural area there seems not to be major problems with Cu and Zn forage content. But soils from Buenos Aires province where grazing systems are established show lower levels of these micronutrients which could be translated in low levels of micronutrient content in forage used as feed for livestock in grazing systems with potential consequences in animal health and nutritional aspects of bovine products as meat.

Forage growth mainly depends on the ability of roots to absorb water and nutrients. Organic fertilization is a method based on the use of manure from cattle and poultry production systems to reduce the application of inorganic commercial fertilizers. Manure contains high levels of nitrogen, phosphorus and organic matter (Barzegar et al., 2006).

In our pot experiments ryegrass was selected due to its frequent occurrence in grazing systems in our country. The trace elements content of beef cattle manure is largely related to their concentration in the feed consumed by livestock (Miller et al., 1991; Nicholson et al., 1999). The main source of trace elements is the mineral core that contains determined concentrations of each element. The percentage of mineral core added to the feed, conditions the trace element content in manure.

In our country, manure from feed lots has a Cu content between 9.2 and 59.6 μg/g and Zn content between 62.4 and 405.7 μg/g (Moscuzza and Fernández-Cirelli, 2009). These micronutrients concentrations were considered in our pot culture experiments.

Fig. 1 shows ryegrass leaf mass (in grams of dry matter) per pot for the first cut (30 days) comparing fertilization techniques (surface and homogenization).

The average of ryegrass leaf mass in blank reactors (no fertilization) was 1.31 ± 0.1 g of dry matter and in the fertilized pots, 1.86 ± 0.08 (surface fertilization) and 1.82 ± 0.06 (fertilization by

Table 2
Copper and zinc content in alfalfa plants from Cordoba province.

Sample	Cu	Zn
1	8.74	36.2
2	15.2	37.3
3	13.5	30.3
4	10.5	26.8
5	9.3	20.2
6	9.8	21.8
7	11.4	31.5
8	13.4	27.1
9	8.5	23.3

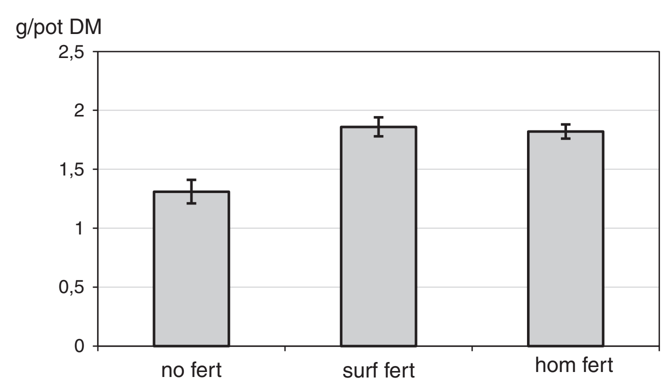

Fig. 1. Ryegrass leaf mass (in g/pot DM) in the first cut comparing fertilized and unfertilized reactors.

homogenization). The difference of ryegrass leaf mass between fertilized and unfertilized reactors was around 40%.

In grazing systems, minerals come from natural pastures and drinking water. An alternative to mineral supplementation could be fertilization of forage with mineral-rich organic amendments. Average concentrations of Cu and Zn found in the ryegrass samples for each tested concentration, and different fertilization procedure, are presented in Table 3.

The results showed significant differences ($P<0.05$) in the concentrations of Cu and Zn between spiked and non spike (Table 4). There were no significant differences ($P>0.05$) in Cu and Zn concentrations considering the fertilization methodology (homogenization or superficial).

In Argentina, Lavado (2006) reported Zn levels in natural grasslands from 35.6 to 42.5 μg/g, and in forage from 27.7 to 32.4 μg/g. Plants with less than 20 μg/g of Zn (dry basis) are deficient in this element; normal values in plants are in the range of 25 to 150 μg/g. In our experiments, Zn levels in plants fertilized with composted manure treated with solution SN1 were in the range reported in literature in the second harvest, while reactors treated with solutions SN2 and SN3 were within this range in the first harvest. Between the first and second harvest we observed an increase in Zn concentration in ryegrass for all tested treatments. Zinc concentrations increased with increasing levels of this element in the enriched excreta. For the second harvest, the plants treated with the three solutions (SN 1, SN2 and SN3) showed Zn levels markedly higher than those reported as deficient. On the other hand, values observed are non-toxic for livestock (Gupta et al., 2008).

Forages generally contain Cu levels between 3 and 8 μg/g dry matter (Minson, 1990). In our experiments, Cu levels in fertilized and unfertilized controls were below the limit of deficiency reported in literature: 5 μg/g dry matter (Gupta and Gupta, 1998). Cu levels in plants fertilized with composted manure treated with solutions SN2 and SN3 showed for the second harvest Cu levels comprised in the range reported and around the limit of deficiency.

The results described above are a promissory alternative to mineral supplementation in grazing systems. The use of composted manure from feed lots should reduce the environmental risk of pollution from the excreted micronutrients and their incorporation as organic soil amendments can be useful to compensate Cu and Zn deficiencies in cattle through forage enrichment.

4. Conclusions

This paper reports micronutrient content in different soils in Argentina, where trace metal studies in environmental matrix are scarce. Natural forage (*Medicago sativa*) was also analyzed. Data concerning the geographical distribution of micronutrients in the environment are essential to establish associations between animal and human health and the environment, useful for epidemiological studies.

Soil–plant transference factors were not estimated, since they are useless due to the number of factors involved, where the soil characteristics play a decisive role in trace metals bioavailability. Soils with similar content of organic matter show different behaviors in trace element transfer to forage, while in other examples higher organic matter induces low transfer to forage.

Moreover, in our pot culture experiments, using the same solid matrix with increasing concentrations of Cu and Zn it can be observed that there is no linear relationship between soil concentration and plant concentration. A logarithm trend tending to a maximum may be visualized.

Cu and Zn enrichment was observed in the more concentrated solutions and the levels obtained in ryegrass are over the limit of deficiency reported in the literature.

Fertilization rate used in the experiments is the recommended one taking into account nitrogen content in manure, and Cu and Zn contents were added taking into account values previously determined in argentine feed lots. Therefore, the results here reported may be extrapolated to the real situation using manure from our feed lot systems.

Table 3
Average concentrations of Cu and Zn in ryegrass cropped in reactors spiked with different Cu and Zn concentrations.

	Harvest 1 [Cu]	Harvest 2	Harvest 1 [Zn]	Harvest 2
Without fertilization	< 1*	2.1 ± 0.02	< 1*	8.3 ± 0.03
Fertilization with homogenization				
SN 0	1.2 ± 0.02	2.2 ± 0.04	10.8 ± 0.06	39.9 ± 0.03
SN 1	2.8 ± 0.01	3.4 ± 0.01	13.9 ± 0.04	56.9 ± 0.08
SN 2	1.8 ± 0.03	4.5 ± 0.06	47.1 ± 0.03	66.3 ± 0.06
SN 3	3.4 ± 0.02	4.9 ± 0.01	44.3 ± 0.09	69.1 ± 0.06
Superficial fertilization				
SN 0	1.5 ± 0.05	2.9 ± 0.01	12.2 ± 0.09	38.1 ± 0.05
SN 1	3.2 ± 0.05	3.7 ± 0.02	20.1 ± 0.03	52.0 ± 0.07
SN 2	2.9 ± 0.08	4.9 ± 0.04	37.6 ± 0.05	68.2 ± 0.1
SN 3	3.8 ± 0.02	5.1 ± 0.05	41.7 ± 0.08	75.3 ± 0.09

* Detection limit.

Table 4
Results of ANOVA on Cu and Zn concentrations (μg/g) between fertilized and unfertilized reactors.

	[Cu]	[Zn]
SN0	$F_{(2,9)}=247.15$**	$F_{(2,9)}=15160.48$**
SN1	$F_{(2,9)}=1863.48$**	$F_{(2,9)}=67538.58$**
SN2	$F_{(2,9)}=2212.53$**	$F_{(2,9)}=270731.5$**
SN3	$F_{(2,9)}=4642.18$**	$F_{(2,9)}=330610.4$**

** $P<0.05$.

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