Effects of amino acids and casein on copper uptake from soil by chicory (*Cichorium intybus* L. cv. Grasslands Puna)

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Abstract

A greenhouse pot trial was conducted to investigate the effect of addition of amino acids and casein to Cu fertilisers on Cu uptake by chicory (*Cichorium intybus* L. cv. Grasslands Puna) plants. Irrespective of the forms and rates of Cu applied, the addition of amino acids and casein had no significant effects on chicory shoot dry weight. $CuSO_4$ resulted in significantly higher shoot Cu accumulation than $Cu(OH)_2$. Addition of histidine (His), methionine (Met), aspartic acid (Asp) and casein to $Cu(OH)_2$ significantly increased Cu uptake at 100 mg Cu/kg soil treatments. Total accumulation of Cu from casein + Cu was significantly higher than from Met + Cu and Cu alone at 50 mg Cu as $CuSO_4$/kg soil treatments. At the rate of 100 mg Cu as $CuSO_4$/kg soil, the addition of His and casein resulted in significantly higher total shoot Cu accumulation than the addition of Met, Asp and $CuSO_4$ alone. When Cu was supplied as $Cu(OH)_2$, addition of His, Met, Asp and casein increased Cu uptake by 2.86–8.85, 3.81–6.19, 3.81–12.39 and 9.52–15.04% respectively. The corresponding values for $CuSO_4$ with the addition of His, Met, Asp and casein were 10.88–14.60, 4.08–4.32, 3.24–6.80 and 20.41–21.08% respectively. These results suggest that the addition of amino acids or casein to Cu fertilisers can increase chicory Cu uptake, but the agronomic value of the mixtures needs further investigation.

Keywords: additives, amino acids, casein, chicory (*Cichorium intybus* L), copper fertilisers, copper uptake

Abbreviations: Asp, aspartic acid; Glu, glutamic acid; His, histidine; Met, methionine; Gly, glycine; NA, nicotianamine; DW, dry weight.

Introduction

Copper deficiency in grazing animals is widespread in New Zealand, costing the farming industry several million dollars in animal remedies each year. In New Zealand, Cu deficiency in ruminants can occur as a result of ingesting pasture containing less than 3 to 4 mg Cu/kg DM and from molybdenum/sulphur induced Cu deficiency (Grace 1983). Recently, Grace *et al.* (1998) found a good correlation between herbage Cu concentration and liver Cu concentration of grazing Romney sheep. Only trace amounts of Cu are required for plants and animals, but the contents of forage plants may sometimes not provide adequate Cu intake for the optimum performance of ruminant animals (Jarvis 1978). Overcoming the problem with Cu fertilisers has met with mixed success, with generally low uptake of Cu by pasture plants. Low Cu uptake is caused by low solubility of plant-available forms of Cu in the soil solution and inefficient transport of Cu from roots to shoots (Jarvis 1978, 1980). An important contribution to the prevention of Cu deficiency in animals would be an effort to increase the Cu concentrations of forage plants. This can be achieved by modest increases in Cu uptake by major pasture grasses or by changing the botanical composition of the forage crop to include species with greater Cu content.

From the results of mathematical models, field experiments and studies with synthetic chelates, Bineev *et al.* (1985) confirmed that free amino acids in soils form chelates with Cu and thereby facilitate the migration of the trace element in the soil-plant system. More recently, it was found that free nicotianamine (NA) and histidine (His) are the major Cu transporters in tomato and chicory xylem sap (Liao *et al.* 1999; Pich & Scholz 1996). Chicory (*Cichorium intybus* L), a forage plant, is recognised as having higher Cu concentration in shoots than many pasture species, and is a valuable source of forage Cu (Thomas *et al.* 1952). This paper tests the hypothesis that Cu uptake in chicory can be increased by addition of amino acids to Cu fertilisers, either by increasing water-soluble Cu in soils or Cu transport from roots to shoots.

Materials and methods

A sample of Manawatu silt loam (2.9% OM, 6.0 pH, 52 µg/g Olsen P, 17 meq/100 g soil CEC, 2 µg/g EDTA extractable Cu) was obtained from a 0–15 cm soil depth under permanent pasture. The soil was air dried and sieved (<5 mm particle size). Sub-samples of 500 g soil were weighed into plastic bags. Cu was applied either as $Cu(OH)_2$ or $CuSO_4$ at 50 mg or 100 mg Cu/kg air-dry soil. His, methionine (Met), aspartic acid (Asp) and casein were used as additives. The amount of amino acids (His, Met, Asp) and casein applied were determined by the amount needed to supply 18 mg organic-N/pot. $(NH_4)_2SO_4$ was supplied to make up the total N application to 50 mg/pot. The Cu fertilisers, amino acids, casein, and $(NH_4)_2SO_4$ were mixed with the dry soil in the plastic bags before placing the soil in the pots.

Even-sized chicory cv. Grassland Puna seedlings were transplanted into the pots. Distilled water was supplied to maintain 80% of pot water capacity. After 10 days, 200 ml of Cu-free complete nutrient solution was added weekly to each pot to supply 40 mg N for plant growth. Plants were harvested at 40, 70 and 90 days after transplanting. Harvested plant materials were rinsed with distilled water, and oven dried at 65°C for 48 hours. Plant dry weight (DW) was recorded. Cu concentrations in plant shoots were measured using flame atomic absorption spectrometry (FAAS) after digesting with 69% HNO_3.

The significance of differences between treatment mean plant dry weights and Cu uptakes was tested using analysis of variance (ANOVA) (SAS Institute, Inc. 1987), and means were separated by LSD.

Results and discussion

Irrespective of the forms and rates of Cu applied, the addition of amino acids and casein had no significant effects on chicory shoot dry weight (data not shown). Over three harvests, the chicory shoot Cu concentrations ranged from 9 to 29 mg Cu/kg DW (Figure 1). No obvious Cu toxicity symptoms were observed in any of the treatments during the experimental period. No Cu deficiency symptoms were observed for the control plants grown in Manawatu silt loam.

Shoot Cu accumulation significantly increased (P values in Table 1) with increased application of Cu (Table 1). This result agrees with that of other workers (Beck 1962; Grace *et al.* 1998; Jarvis 1978, 1980). Applications of $CuSO_4$ fertiliser resulted in significantly higher shoot Cu accumulation than $Cu(OH)_2$ (Table 1), reflecting the lower solubility of $Cu(OH)_2$.

When Cu was supplied as $Cu(OH)_2$ at 50 mg Cu/kg soil, there were no significant increases in shoot Cu concentration above the control pot (Figure 1). At 100 mg Cu/kg soil addition, $Cu(OH)_2$ alone significantly increased shoot Cu concentration above the concentrations measured in the control pots. At harvest III, all amino acid and protein additives significantly increased shoot Cu concentration compared to control plant and $Cu(OH)_2$ alone (Figure 1), but there were no significant differences among additives. This result is also reflected in the Cu uptake values reported in Table 2.

Application of $CuSO_4$ significantly increased shoot Cu concentration compared to control plants. The casein + $CuSO_4$ treatment resulted in significantly higher shoot Cu concentration than the treatment without additive ($CuSO_4$ alone) at harvest III at 50 mg Cu addition and

Figure 1 The effect of Cu source and amount applied on shoot Cu concentration of chicory plants grown in pots of Manawatu silt loam. A: 50 mg Cu as $Cu(OH)_2$/kg soil; B: 100 mg Cu as $Cu(OH)_2$/kg soil; C: 50 mg Cu as $CuSO_4$/kg soil; D: 100 mg Cu as $CuSO_4$/kg soil. NS indicates no significant difference. The vertical bars are the $LSD_{0.05}$ values calculated from ANOVA analysis. Significant differences (P<0.05) between treatment means at a single harvest are signified by different letters.

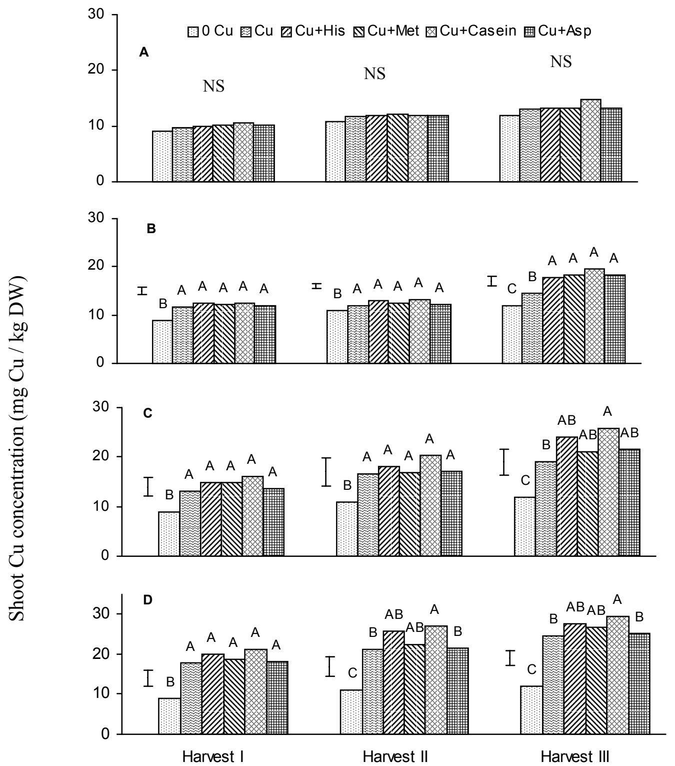

Table 1 Effects of Cu fertiliser source and amount applied on accumulated shoot dry weight, and shoot Cu accumulation of chicory plants. Data are means of three replicates.

	------------ Shoot Cu uptake/pot (mg) ------------			Total shoot dry weight/ pot (g)	Total shoot Cu uptake/ pot (mg)
	Harvest I	Harvest II	Harvest III		
Cu form					
$Cu(OH)_2$	0.041 a	0.039 a	0.036 a	9.27	0.115 a
$CuSO_4$	0.065 b	0.063 b	0.053 b	9.07	0.181 b
SE[1]	0.003	0.002	0.002	0.16	0.003
P	0.05	0.01	0.05	NS	0.01
Cu level (mg/kg soil)					
0	0.035 a	0.040 a	0.026 a	9.47	0.102 a
50	0.049 b	0.045 a	0.039 b	9.32	0.132 b
100	0.054 c	0.056 b	0.048 c	9.03	0.158 c
SE[1]	0.003	0.003	0.002	0.34	0.005
P	0.01	0.05	0.01	NS	0.01

Pool data are analysed.
[1]SE: Standard error of mean.
Within a harvest, results of LSD tests are indicated either as not significant (NS) or at the probability level (P) stated, if significant.

Table 2 The effect of Cu source, amount applied and additives on accumulated biomass and accumulated Cu uptake. Data shown are means of three replicates. Results of LSD tests are indicated either as not significant (NS), or significantly different when means are labeled with different letters.

--------------------- Treatment --------------------- Cu source	additive	Accumulated biomass (g)	Accumulated Cu uptake (mg)	Efficiency of additive (%)
50 mg Cu as $Cu(OH)_2$/kg soil	NL[1]	9.46	0.105	
	His	9.41	0.108	2.86
	Met	9.45	0.109	3.81
	Asp	9.44	0.109	3.81
	Casein	9.58	0.115	9.52
	P	NS	NS	
100 mg Cu as $Cu(OH)_2$/kg soil	NL	9.11	0.113 a	
	His	9.12	0.123 b	8.85
	Met	8.92	0.120 b	6.19
	Asp	9.28	0.127 b	12.39
	Casein	8.99	0.130 b	15.04
	P	NS	0.05	
50 mg Cu as $CuSO_4$/kg soil	NL	9.25	0.147 a	
	His	9.13	0.163 ab	10.88
	Met	9.08	0.153 a	4.08
	Asp	9.34	0.157 ab	6.80
	Casein	9.06	0.177 b	20.41
	P	NS	0.05	
100 mg Cu as $CuSO_4$/kg soil	NL	9.00	0.185 a	
	His	8.88	0.212 b	14.60
	Met	8.83	0.193 a	4.32
	Asp	9.13	0.191 a	3.24
	Casein	8.91	0.224 c	21.08
	P	NS	0.01	

[1]NL: no additive

at harvest II and harvest III at 100 mg Cu addition (Figure 1). Total Cu uptake from casein + Cu was significantly higher than from Met + Cu and $CuSO_4$ alone (Table 2). At the rate of 100 mg Cu/kg soil as $CuSO_4$, the addition of His and casein resulted in significantly higher total shoot Cu accumulation than Met, Asp and $CuSO_4$ alone. The total shoot Cu accumulation of the casein + Cu treatment was significantly higher than the His + Cu treatment (Table 2).

The effect of additives on chicory Cu uptake, expressed as efficiency of additives ((Cu uptake with additives – Cu uptake without additives)/Cu uptake without additive × 100) is presented in Table 2. When

Cu was supplied at 50 mg Cu as $Cu(OH)_2$, addition of His, Met, Asp and casein increased Cu uptake by 2.86, 3.81, 3.81 and 9.52% respectively. The corresponding values for 50 mg Cu as $CuSO_4$ with addition of His, Met, Asp and casein were 10.88, 4.08, 6.80 and 20.41% respectively. When Cu was supplied at 100 mg Cu as $Cu(OH)_2$, addition of His, Met, Asp and casein increased Cu uptake by 8.85, 6.19, 12.39 and 15.04% respectively. The corresponding values for 100 mg Cu as $CuSO_4$ with addition of His, Met, Asp and casein were 14.60, 4.32, 3.24 and 21.08% respectively. Bineev *et al.* (1985) found a similar result for rye by applying Met and Gly to a gray forest soil. They found that the total content of amino acids in soil increased as a result of enhanced microbial activity. Free amino acids in soil form chelates with Cu which facilitate the migration of the trace element in the soil-plant system.

Conclusions and implications

Under glasshouse conditions, initial Cu uptake by chicory plants from soil is more rapid from $CuSO_4$ than $Cu(OH)_2$ fertiliser because of the solubility differences between the sources. The initial uptake of Cu from $CuSO_4$ fertilised soil can be increased by 10–21% by addition of His and casein. Casein was generally more effective at increasing plant Cu uptake than His and other amino acids. The results indicate that the agronomic value of amino acid- or protein-based Cu fertiliser may warrant further investigation, particularly aspects of granular fertiliser development and their Cu release rates.

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