The Effects of Copper Source and Concentration on Lipid Metabolism in Growing and Finishing Angus Steers*

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ABSTRACT : Forty-eight individually fed Angus steers (body weight 220 kg±9.1) were utilized to investigate the effects of copper (Cu) source and concentration on lipid metabolism and carcass quality. Steers were stratified by body weight and initial liver Cu concentration and randomly assigned to one of five groups. Groups were then randomly assigned to treatments. Treatments consisted of: 1) control (no supplemental Cu); 2) 10 mg Cu/kg DM from $CuSO_4$; 3) 10 mg Cu/kg DM from a Cu amino acid complex (Availa Cu) 4) 20 mg Cu/kg DM from $CuSO_4$; and 5) 20 mg Cu/kg DM from Availa Cu. Steers were fed a corn-alfalfa-based growing diet for 56 d. Steers were then switched to a high concentrate finishing diet for 145 d. On day 74 of the finishing phase subcutaneous adipose tissue biopsies were obtained from three steers/treatment to determine basal and stimulated lipolytic rates *in vitro*. Steers were then slaughtered after receiving the finishing diet for 145 d. Control steers tended ($p<0.12$) to have lower ceruloplasmin (Cp) activity than Cu supplemented steers. Steers receiving 20 mg Cu/kg DM from Availa Cu had higher ($p<0.03$) Cp activity than steers receiving 20 mg Cu/kg DM from $CuSO_4$. Plasma non-esterified fatty acids were similar across treatments. Steers receiving 10 mg Cu/kg DM from Availa Cu had higher ($p<0.02$) total plasma cholesterol concentrations relative to steers receiving 10 mg Cu/kg DM from $CuSO_4$. Steers receiving 20 mg Cu/kg DM from Availa Cu had lower ($p<0.03$) plasma triglyceride concentrations than steers supplemented with 20 mg Cu/kg DM from $CuSO_4$. Fatty acid profile of longissimus muscle was similar across treatments. Backfat depth tended ($p<0.18$) to be lower in Cu supplemented steers relative to controls. Steers supplemented with 20 mg Cu/kg DM from Availa Cu had heavier ($p<0.03$) hot carcass weights and a greater ($p<0.02$) dressing percentage than steers supplemented with 20 mg Cu/kg DM from $CuSO_4$. Furthermore, *in vitro* basal ($p<0.06$) and epinephrine stimulated ($p<0.04$) lipolytic rates of subcutaneous adipose tissue were higher in Cu supplemented steers relative to controls. The results of this study suggest that Cu supplementation has minimal effects on blood and lean tissue lipid profile. However, it appears that Cu may play a role in lipid metabolism in subcutaneous adipose tissue. ***(Asian-Aust. J. Anim. Sci. 2003. Vol 16, No. 8 : 1131-1136)***

Key Words : Steers, Copper, Lipid Metabolism, Fatty Acid

INTRODUCTION

Copper (Cu) is an essential element required by ruminants and other animals for many biochemical functions (Davis and Mertz, 1987). Feeding pharmacological concentrations of Cu (250 mg Cu/kg DM) well above dietary requirements has altered lipid metabolism in several animal species. Copper supplementation at high concentrations to diets adequate in Cu decreased breast muscle cholesterol concentrations in poultry (Pesti and Bakali, 1996) and increased the unsaturated fatty acid content of subcutaneous adipose tissue in swine (Amer and Elliot, 1973). High dietary Cu has also been reported to increase fatty acid unsaturation and decrease stearic acid composition of calf liver and heart (Jenkins and Kramer, 1989).

Recent research suggests that feeding physiological concentrations of Cu alters lipid metabolism in ruminants. Copper supplementation (ranging from 10-40 mg Cu/kg DM) to high concentrate finishing diets (marginal in Cu) fed to steers, decreased subcutaneous adipose tissue deposition (Ward and Spears, 1997; Engle et al., 2000a; Engle and Spears, 2000b) and increased polyunsaturated fatty acid composition and tended to decrease cholesterol concentrations of longissimus muscle (Engle et al., 2000a). Furthermore, Sinnett-Smith and Woolliams (1987) reported that Cu supplementation to Cu deficient sheep increased in vitro lipolytic rates of subcutaneous adipose tissue. Therefore, the present study was conducted to confirm the effect of dietary Cu on lipid metabolism in cattle as well as to investigate the effects of Cu concentration and source on subcutaneous adipose tissue lipid metabolism.

MATERIALS AND METHODS

Forty-eight Angus steers (approximately 7 mo age; body weight 220 kg±9.1) from the Colorado State University (CSU) Beef Improvement Center (Saratoga, WY) were utilized in this experiment. Care, handling and sampling of animals herein were approved by the Colorado State University Animal Care and Use Committee. Animals were transported approximately 200 km to the feedlot at the CSU Agriculture Research, Development and Education Center (Fort Collins, CO). Upon arrival, steers were weighed,

* Use of trade names in this publication does not imply endorsement by Colorado State University or criticism of similar products not mentioned.

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Table 1. Ingredient composition of basal diet

Ingredient	Growing	Finishing
	----------%[a]----------	
Whole corn	47	86
Alfalfa hay	45	6
Protein supplement	6.98	6.70
Vitamin premix[b]	0.02	0.03
Mineral premix[c]	1.0	1.0
Protein supplement composition		
Sunflower meal 32%	80.51	-
Ground corn	-	49.04
Limestone	7.00	12.28
Urea	6.00	15.83
Feather meal	2.65	-
Salt	2.08	2.10
Biofos	1.05	7.85
Niacin 98%	0.27	0.25
Tylan 40	0.23	0.16
Rumensin 80[d]	-	0.26
Dyna-K	0.15	6.50
Selenium	0.06	0.08
Wheat midds	-	5.18
Sulfur flowers	-	0.47

[a] Dry matter basis.
[b] Contained per kilogram of diet: 6.000.000 IU of vitamin A, 1.520.000 IU of vitamin D and 6, 600 IU of vitamin E.
[c] Provided per kilogram of diet: 30 mg of Zn as $ZnSO_4$, 20 mg of Mn as $MnSO_4$, 0.5 mg of I as EDDI and 0.1 of Co as $CoCO_3$.
[d] Provided 33 mg of monensin/kg DM.

vaccinated with Bovi-Shield 4+/L5 (Pfizer Animal Health, Exton, PA) and Cattlemaster 4 (Pfizer Animal Health, Exton, PA), treated for parasites with Dectomax (Pfizer Animal Health, Exton, PA) and confined in a group pen for 7 d where they were fed a grass hay diet (5 kg DM/hd/d). Steers were then housed in individual feedlot pens (2 m×12.5 m) equipped with automatic water troughs and cement feed bunks and fed a grass hay diet (6 kg DM/hd/d) for 5 d.

After adjusting to the individual pen feeding system, steers were weighed on two consecutive days, implanted with Ralgro (Scheng Canada Inc., Intervet, Millsboro, DE), bled via jugular venipuncture, and a liver biopsy obtained (Engle and Spears, 2000a) and analyzed for Cu. Steers were stratified by body weight and initial liver Cu concentration and randomly assigned to one of five groups. Groups were then randomly assigned to treatment. Treatments consisted of: 1) control (no supplemental Cu; n=10 steers); 2) 10 mg Cu/kg DM from Cu sulfate ($CuSO_4$; n=10 steers); 3) 10 mg Cu/kg DM from a Cu amino acid complex (Availa Cu; Zinpro Corporation, Eden Prairie, MN, USA; n=10 steers); 4) 20 mg Cu/kg DM from $CuSO_4$ (n=9 steers); and 5) 20 mg Cu/kg DM from Availa Cu (n=9 steers).

Steers were fed a corn-alfalfa-based growing diet for 56 d (Table 1; basal diet contained 7.1 mg Cu/kg DM, 32.8 mg Zn/kg DM, 0.59 mg Mo/kg DM, 68.1 mg Fe/kg DM and 0.14% S). Diets were formulated to meet or exceed all nutrient requirements for growing steers with the exception of Cu (NRC, 1996). Diets were fed once daily, in amounts to allow ad libitum access to feed. Daily feed offerings were recorded and feed refusals were measured every 28 d throughout the experiment in order to calculate feed intake and feed efficiency. On day 0, 28 and 56 of the growing phase steers were weighed and bled via jugular venipuncture. Jugular blood samples were collected in heparinized trace mineral free Vacutainer tubes (Becton Dickenson, Franklin Lakes, NJ) for the determination of plasma ceruloplasmin (Cp) activity (an indicator of Cu status) and triglyceride, total cholesterol and non-esterified fatty acid (NEFA) concentrations. All blood samples obtained throughout the study were taken in the morning prior to the animals being fed. Blood samples were transported to the laboratory on ice, centrifuged at 1,850×g at 5°C for 20 min and plasma was obtained. Plasma was frozen at -20°C until analyzed.

At the end of the growing phase, steers were re-implanted with Revalor S (Intervet, Millsboro, DE) and gradually switched to a high concentrate finishing diet over a 14 d period (Table 1; basal diet contained 6.1 mg Cu/kg DM, 42.3 mg Zn/kg DM, 0.42 mg Mo/kg DM, 49.7 mg Fe/kg DM, and 0.19% S). Steers remained on the same dietary treatments that they received in the growing phase and were bled at 28 d intervals throughout the remainder of the study. On d 74 of the finishing phase adipose tissue biopsies were collected from 15 steers (three steers per treatment) as described by Martin et al. (1999) with modification. Briefly, biopsies were obtained from the right side of the tail head. The area was clipped of hair, scrubbed three times with betadine and 70% ethyl alcohol and the area locally anesthetized with 5 mL of a 2% lidocaine HCl solution (20 mg /mL; Vedco, St Joseph, MO). An incision of approximately 4 cm in length and 2 cm in depth was made at a 30 to 45° angle from the caudal vertebrae. From the incision, a section of adipose tissue weighing approximately 5 g was excised. The incision was then sutured with sterile #2.0 catgut suture and treated with a topical antibiotic agent (Penalog). Adipose tissue samples were rinsed in a physiological buffered saline solution (0.01 M; pH 7.4) to remove excess blood, blotted dry and weighed. Basal and epinephrine stimulated lipolytic rates were determined in vitro within 2 min post-biopsy.

On d 145 of the finishing phase steers were harvested. Weights were taken on two consecutive days and steers were transported approximately 50 km to a commercial abattoir and slaughtered. Hot carcass weight was determined on the day of slaughter. Standard carcass measurements were obtained 48 h post harvesting by a certified USDA grader. Post grading, a thin slice of longissimus muscle tissue was obtained from the 12th and 13th rib interface (approximately 100 g). Longissimus muscle samples were stored in individual plastic bags,

placed on ice, transported to the laboratory and frozen at -80°C until analyzed for total lipid and fatty acid composition.

Analytical procedures

Dietary mineral concentrations were determined via atomic absorption spectrophotometry (Varian Model 1275; as described by Engle et al., 1997). Plasma Cp activity was analyzed according to the procedure described by Houchin (1958). The remaining plasma was analyzed for total cholesterol, NEFA, and triglycerides (TG) via enzymatic and colormetric methods (Sigma Chemical Co., 2000, Wako Chemical Co., 1995, and Sigma Chemical Co., 1990; respectively).

Subcutaneous adipose tissue samples were immediately dissected into six 200 mg segments upon collection. Three segments were placed into a basal buffering solution as described by Pothoven et al. (1975). The remaining three segments were placed in a buffering solution containing 1.5 μg of epinephrine in order to stimulate lipolysis (Pothoven et al., 1975). Samples were incubated for one hour and tissue was then reweighed post-incubation. The buffering solution was decanted and analyzed for glycerol concentrations (Laurell and Tibbling, 1966) in order to determine basal and epinephrine stimulated lipolytic rates.

Longissimus muscle was dissected of visible external fat. The remaining tissue was finely diced until a heterogeneous sample was obtained. Duplicate representative subsamples (1 g) were removed for lipid extraction according to the procedure described by Bligh and Dyer (1959) using a 2:1 mixture of chloroform: methanol. The lipid fraction was dried under nitrogen gas and then placed in a 100°C oven for 4 h. Vials were cooled in a desiccator and their weights were compared to the dry weights of the empty vials to determine percent lipid extracted from the longissimus muscle.

Duplicate longissimus muscle lipid extracts were analyzed for fatty acid composition. Fatty acid composition of the longissimus muscle was determined via gas chromatography using a Hewlett Packard (Avondale, PA) Model 6890 series II gas chromatograph fixed with a series 7683 injector and flame ionization detector. The instrument was equipped with a 100 m×0.25 mm (id) fused silica capillary column (SP-2560 Supelco Inc. Bellefonte, PA). Methyl ester derivatives of fatty acids were prepared using a combination of $NaOCH_3$ followed by HCl/methanol as described by Kramer et al. (1997). Fatty acid methyl ester preparations were injected (1 μL) using the split mode. The carrier gas was helium and the split ratio was 100:1 at 180°C. The oven temperature was programmed from an initial temperature of 140°C (0 min) to a final temperature of 225°C at the rate of 2.8°C/min. The final temperature was held for 18 min. Chromatograms were recorded with a computing integrator (Agilent Technologies, Palo Alto, CA). Standard fatty acid methyl ester mixtures were used to calibrate the gas chromatograph system using reference standards KEL-FIM-FAME-5 (Matreya Inc., PA). Identification of the fatty acids was made by comparing the relative retention times of fatty acid methyl ester peaks from samples with those of known standards. These were calculated as normalized area percentages of fatty acids.

Statistical analysis

Statistical analyses of data were conducted using the Mixed procedures of SAS (SAS Inst. Cary, NC). Model statements included treatment, time, and treatment by time interactions when repeated measurements were taken. However, there were no significant interactions and least squares means for response variables are reported as overall treatment means. Carcass data were adjusted using final body weight as a covariate in order to distinguish between increased weight responses (see Dorton et al., 2002) and treatment effects. When treatment was significant, differences among means were determined using preplanned single degree of freedom contrasts. Comparisons made were: 1) control vs. Cu supplemented

Table 2. Effects of copper source and concentration on blood lipid concentrations and ceruloplasmin activity

Variable	Treatment					SEM	Contrasts (p<)			
	Control	10 ppm $CuSO_4$	10 ppm Availa Cu	20 ppm $CuSO_4$	20 ppm Availa Cu		Conrtol vs. Copper	10 ppm vs. 20 ppm	$CuSO_4$ vs. Availa Cu at 10 ppm	$CuSO_4$ vs. Availa Cu at 20 ppm
Total cholesterol, mg/dL	75.3	75.8	88.5	80.6	79.4	3.90	0.18	0.58	0.02	0.84
Non-esterified fatty-acids, mmol/L	0.22	0.22	0.21	0.22	0.19	0.02	0.53	0.80	0.65	0.25
Triglycerides, mg/dL	22.6	22.5	21.5	22.3	21.0	0.40	0.09	0.47	0.09	0.03
Ceruloplasmin (AU)	0.15	0.15	0.16	0.16	0.17	0.004	0.12	0.17	0.4	0.03

Table 3. Effects of copper source and concentration on carcass characteristics of finished steers[a]

Variable	Treatment					SEM	Contrasts (p<)			
	Control	10 ppm $CuSO_4$	10 ppm Availa Cu	20 ppm $CuSO_4$	20 ppm Availa Cu		Conrtol vs. Copper	10 ppm vs. 20 ppm	$CuSO_4$ vs. Availa Cu at 10 ppm	$CuSO_4$ vs. Availa Cu at 20 ppm
12th rib backfat (cm)	1.45	1.30	1.45	1.18	1.35	0.085	0.18	0.18	0.24	0.16
Marbling[b]	412.7	406.0	442.8	379.5	386.5	24.50	0.74	0.10	0.30	0.84
LMA[c] (cm^2)	74.3	70.8	75.0	71.0	73.1	1.9	0.37	0.66	0.12	0.42
Hot carcass weight	322.6	321.2	326.0	318.9	326.2	2.3	0.85	0.66	0.15	0.03
Dressing percent	58.4	58.1	59.0	57.7	59.1	0.004	0.87	0.69	0.13	0.02
KPH[d] fat (%)	1.8	1.9	1.9	2.0	2.0	0.07	0.15	0.10	0.78	0.71

[a] Data were adjusted using initial body weight as a covariate. [b] 300=slight; 400=small; 500=modest. [c] Longissimus muscle area. [d] Kidney, pelvic and heart fat.

steers. 2) 10 mg Cu/kg DM vs. 20 mg Cu/kg DM. 3) 10 mg Cu/kg DM from $CuSO_4$ vs. 10 mg Cu/kg DM from Availa Cu. and 4) 20 mg Cu/kg DM from $CuSO_4$ vs. 20 mg Cu/kg DM from Availa Cu.

RESULTS AND DISCUSSION

During the study one steer from the 10 mg Cu/kg DM from $CuSO_4$ treatment died from bloat and all data pertaining to this animal were removed from statistical analysis. Steer performance data and plasma and liver mineral concentrations are reported elsewhere (Dorton et al., 2002). Briefly, feed intake, feed efficiency, and average daily gain (ADG) were similar between Cu supplemented steers and controls during both the growing and the finishing phases. However, overall ADG tended ($p<0.10$) to be higher and final body weight was higher ($p< 0.05$) in steers supplemented with 10 mg Cu/kg DM from Availa Cu compared to steers supplemented with 10 mg Cu/kg DM from $CuSO_4$.

There was an overall treatment effect ($p<0.05$) for plasma Cp activity. Control steers tended ($p<0.12$) to have lower plasma Cp activity than Cu supplemented steers (Table 2). Steers receiving 20 mg Cu/kg DM from Availa Cu had higher ($p<0.03$) plasma Cp activities than steers receiving 20 mg Cu/kg DM from $CuSO_4$.

Blood lipid composition was comprised of plasma total cholesterol, NEFA and TG (Table 2). Plasma NEFA concentrations were similar across treatments. However, Cu supplemented steers tended ($p<0.18$) to have higher plasma total cholesterol concentrations than control steers. Steers receiving 10 mg Cu/kg DM from Availa Cu had higher ($p<0.02$) total plasma cholesterol concentrations than steers receiving 10 mg Cu/kg DM from $CuSO_4$. These results contradict findings by Engle and Spears (2000b) who reported lower plasma cholesterol concentrations in Cu supplemented steers relative to controls. It is unclear as to why this discrepancy exists. Although supplemental Cu concentrations were similar between the two studies, Cu-supplemented steers in the study conducted by Engle and Spears (2000b) had higher plasma Cu concentrations (0.94 mg Cu/L) than Cu supplemented steers in the present study (0.79 mg of Cu/L). This suggests that the plasma Cu concentrations for the current experiment may not have been elevated to levels that stimulate a reduction in plasma cholesterol concentrations. The reason for the difference in plasma Cu concentrations may be due to differences in basal diet composition, antagonists present in the basal diet, or certain environmental factors between experiments.

Plasma TG concentrations tended ($p<0.09$) to be lower in Cu supplemented steers relative to the controls (Table 2). Steers receiving 10 mg Cu/kg DM from Availa Cu tended ($p<0.09$) to have lower plasma TG concentrations than steers supplemented with 10 mg Cu/kg DM from $CuSO_4$. Furthermore, steers receiving 20 mg Cu/kg DM from Availa Cu had lower ($p<0.03$) plasma TG concentrations relative to steers supplemented with 20 mg Cu/kg DM from $CuSO_4$. Engle et al. (2000a) reported no difference in plasma TG concentrations between Cu supplemented and control steers. However, Cu supplemented broiler chickens had lower plasma TG concentration than controls (Bakalli et al., 1995).

Carcass characteristics are shown in Table 3. Data were adjusted using final live body weight as a covariant to account for body weight differences between Cu treatment groups as reported by Dorton et al. (2002). Steers receiving 20 mg Cu/kg DM tended ($p<0.10$) to have lower marbling scores than steers supplemented with 10 mg Cu/kg DM.

Subcutaneous adipose tissue (backfat) thickness tended ($p<0.18$) to be lower in Cu supplemented steers compared to controls. There was a similar trend ($p<0.18$) for a lower backfat thickness in steers supplemented with 20 mg Cu/kg DM compared to steers supplemented with 10 mg Cu/kg DM. These data are similar to results from previous research (Ward and Spears, 1997; Engle et al., 2000a, b, c).

Copper supplemented steers tended ($p<0.15$) to have a higher kidney, pelvic and heart (KPH) fat percentage relative to controls. It appears that Cu has different effects on different adipose tissue depot sites (backfat vs. KPH fat).

Table 4. Effects of copper concentration and source on basal and stimulated rates of lipolysis in subcutaneous adipose tissue in finishing steers

Item	Treatment					SEM	Contrasts (p<)			
	Control	10 ppm $CuSO_4$	10 ppm Availa Cu	20 ppm $CuSO_4$	20 ppm Availa Cu		Control vs. Cu	10 mg Cu vs. 20 mg Cu	10 $CuSO_4$ vs. 10 Availa Cu	20 $CuSO_4$ vs. 20 Availa Cu
	μ moles of glycerol released g tissue^{-1} h^{-1}									
Basal lipolysis	0.59	0.74	0.75	0.79	0.81	0.08	0.06	0.21	0.31	0.17
Epinephrine stimulated lipolysis	1.34	1.59	1.60	1.65	1.72	0.11	0.04	0.27	0.28	0.16

Lipolysis, fatty acid synthesis rates and lipogenic gene expression have been reported to differ between adipose tissue locations (Pothoven et al., 1975; Mobi et al., 2000). Therefore, it is possible that different adipose tissue depot sites respond differently to dietary Cu supplementation. Steers receiving 10 mg Cu/kg DM tended ($p<0.10$) to have lower KPH fat when compared to steers supplemented with 20 mg Cu/kg DM.

Steers supplemented with 10 mg Cu/kg DM from Availa Cu tended ($p<0.12$) to have larger longissimus muscle areas, hot carcass weights (HCW; $p<0.15$) and dressing percentages ($p<0.13$) than steers supplemented with 10 mg Cu/kg DM from $CuSO_4$. Steers supplemented with 20 mg Cu/kg DM from Availa Cu had higher HCW ($p<0.03$) and dressing percentage ($p<0.02$) than steers supplemented with 20 mg Cu/kg DM from $CuSO_4$

Percent lipid and fatty acid profile of longissimus muscle were similar across treatments (data not shown). This is in contrast to previously reported research. Engle and Spears (2000b) reported an increase ($p<0.05$) in the unsaturated:saturated fatty acid ratio in longissimus muscle of Cu supplemented steers. This discrepancy may be, in part, due to differences in basal dietary ingredients between the two studies. The basal diet utilized by Engle and Spears (2000b) contained soybean meal, whereas no soybean products were used in the basal diet in the present study. Soybean products have been reported to decrease dry matter digestibility and inhibit complete biohydrogenation in the rumen, thus contributing to the greater occurrence of unsaturated fatty acids and conjugated dienes in tissue (Noble et al. 1974; Whitney et al., 2000). The basal diet of the present study did not contain soybean products and no change in fatty acid composition of longissimus muscle was observed. It is possible that soybean products need to be present in the diet in combination with Cu in order to alter fatty acid composition in tissues.

Basal and epinephrine stimulated lipolytic rates of subcutaneous adipose tissue are shown in Table 4. Copper supplementation increased basal ($p<0.06$) and epinephrine stimulated ($p<0.04$) lipolytic rates in subcutaneous adipose tissue when compared to controls. This is in agreement with earlier research where Cu supplementation to Cu deficient sheep increased basal and stimulated lipolytic rates of subcutaneous adipose tissue (Sinnett-Smith and Woolliams, 1987). Copper is thought to have its affect on lipolysis by increasing cell size and volume (Sinnett-Smith and Woolliams, 1987) as well as being a component of the enzyme dopamine β-hydroxylase (Murray et. al., 2000). Dopamine β-hydroxylase is a cuproenzyme that catalyzes the conversion of dopamine to norepinephrine, a lipolytic hormone (Prohaska et al., 1990). Copper supplementation has been shown to affect catecholamine metabolism in rats (Prohaska and Wells, 1974), sheep (O'Dell et al., 1976), and cattle (Engle et al., 2000b). These findings, indicating increased lipolytic rate with Cu supplementation, further support the trends seen for a reduction in backfat in Cu supplemented steers. Copper concentration (10 or 20 mg Cu/kg DM) or source had no effect on basal or simulated lipolytic rates.

IMPLICATIONS

The results of this study indicate that Cu supplementation had minimal effects on blood lipid concentrations and longissimus muscle fatty acid profile. However, it appears that Cu may play a role in the lipid metabolism of subcutaneous adipose tissue. Further research is needed to determine the metabolic role of Cu in subcutaneous adipose tissue metabolism.

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