Asian-Aust. J. Anim. Sci.
Vol. 23, No. 5 : 573 - 579
May 2010

www.ajas.info

The Effects of Different Copper (Inorganic and Organic) and Energy (Tallow and Glycerol) Sources on Growth Performance, Nutrient Digestibility, and Fecal Excretion Profiles in Growing Pigs

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ABSTRACT : This study was conducted to determine the effects of different copper (inorganic and organic) and energy (tallow and glycerol) sources on growth performance, nutrient digestibility, gas emission, diarrhea incidence, and fecal copper concentration in growing pigs by using a 2×2 factorial design. In this trial, 96 pigs (63 d of age) were employed, with an average initial weight of 28.36±1.14 kg. The dietary treatments were i) basal diet with 134 ppm copper (Korea recommendation) as $CuSO_4$+tallow; ii) basal diet with 134 ppm Cu as $CuSO_4$+glycerol; iii) basal diet with 134 ppm copper as CuMet+tallow; and iv) basal diet with 134 ppm copper as CuMet+ glycerol. Throughout the entire experimental period, no differences were noted among treatment groups with regard to the magnitude of improvement in ADG (average daily gain), ADFI (average daily feed intake) and G/F (gain:feed) ratios. The nitrogen (N) digestibility of pigs fed on diets containing organic copper was improved as compared with that observed in pigs fed on diets containing inorganic copper ($p<0.05$). An interaction of copper×energy was observed in the context of both nitrogen ($p<0.05$) and energy ($p<0.01$) digestibility. Ammonia emissions were significantly lower in the organic copper-added treatment groups than in the inorganic copper-added treatment groups ($p<0.05$). Mercaptan and hydrogen sulfide emissions were reduced via the addition of glycerol ($p<0.05$). No significant effects of copper or energy source, or their interaction, were observed in reference to diarrhea appearance and incidence throughout the entirety of the experimental period. The copper concentration in the feces was significantly lower in the organic copper source treatment group than was observed in the inorganic copper source treatment group ($p<0.05$). The results of this experiment show that organic copper substituted for inorganic copper in the diet results in a decreased fecal copper excretion, but exerts no effect on performance. The different energy (tallow and glycerol) sources interact with different copper sources and thus influence nutrient digestibility. Glycerol supplementation may reduce the concentrations of odorous sulfuric compounds with different Cu sources. (**Key Words :** Growing Pig, Copper, Energy Source, Digestibility, Fecal Excretion)

INTRODUCTION

Feeding pig diets containing 125 to 250 ppm of copper in the form of copper sulfate ($CuSO_4$) has been demonstrated to improve performance in swine (Stahly et al., 1980; Roof and Mahan, 1982), however, the copper requirement by NRC (1998) for pigs is only 5 to 6 ppm. When administered at a concentration of 100 to 250 ppm, this element has been shown to be effective for growth promotion with antibacterial action in the pig (Braude, 1967; Cromwell et al., 1981), due to the fact that dietary copper functions in an antibiotic-like fashion, by influencing microbial growth within the intestinal tract (Bunch et al., 1965; Cromwell, 2001). Feeding with high levels of dietary copper results in an increase in the concentration of copper in manure, which is then applied to soil, which poses a potential environmental threat (Kornegay and Verstegen, 2001). Moreover, the desired antimicrobial effect of high level dietary copper in the intestinal tract results in similar undesirable effects on the bacteria associated with waste degradation in lagoons (Gilley et al., 2000). Restrictions on the addition of trace minerals to livestock diets are already being implemented in Europe, thereby resulting in the necessity for more environmentally conscious alternatives to increase productivity in swine operations. Nowadays, the allowable copper levels in the diets of growing pigs has been decreased from 250 to 134 ppm in Korea, because the typical regimen used in stockbreeding programs worldwide is tending toward a decrease in the amounts of copper

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Received August 6, 2008; Accepted July 30, 2009

supplementation utilized, and the introduction of more bioavailable copper sources.

The previous research conducted by Dove and Haydon (1992) and Dove (1995) showed that weanling pigs at approximately 26 d of age were unable to utilize fat efficiently when fed on basal diets (5 or 15 ppm Cu) during the first 14 d or 21 d of postweaning; however, the addition of 250 ppm Cu improved the digestibility and utilization of the fat in these diets.

The production of diesel fuel from vegetable oil has exponentially increased in the United States, from less than 2 million liters in 1999 to almost 1 billion liters in 2005 (National Biodiesel Board, 2007). Via the biodiesel production reaction, the fatty acids are methylated to form methyl alkyl esters (i.e., biodiesel), and crude glycerol is the principal coproduct from this process (Ma and Hanna, 1999; van Gerpen, 2005; Thompson and He, 2006). Studies assessing the effects of feeding chemically pure or crude glycerol from biodiesel production to broiler chickens (Simon et al., 1996; Cerrate et al., 2006), turkey hens (Rosebrough et al., 1980), and pigs (Kijora et al., 1995) have demonstrated that glycerol can be used as a source of dietary energy for livestock.

Therefore, the principal objective of this study was to determine the effects of different copper sources (inorganic and organic) and different energy sources (tallow and glycerol) on the growth performance, nutrient digestibility, fecal pH, gas emissions, diarrhea incidence, and fecal copper concentration in nursery pigs.

MATERIALS AND METHODS

Experimental design, animals, housing and diets

A total of 96 ((Landrace×Yorkshire)×Duroc) pigs with an average initial BW of 28.36±1.14 kg (63 d of age) were employed in the current experiment in an effort to determine the effects of different sources of dietary energy (tallow and glycerol) and dietary copper (organic and inorganic) on performance, nutrient digestibility, gas emission, diarrhea incidence, and fecal copper concentration in growing pigs. There are 6 replicate pens with 4 pigs per pen and each pen has two barrows and two gilts. The experiments were conducted for 35 days at the Dankook university's swine research farm. At the beginning of the experiment, the pigs were divided into four treatment groups, with six replicate pens per treatment and four pigs per pen, in accordance with a completely randomized design based on the BW. The four treatments administered were as follows: i) basal diet with 134 ppm copper as $CuSO_4$+tallow; ii) basal diet with 134 ppm copper as $CuSO_4$+glycerol; iii) basal diet with 134 ppm copper as CuMet+tallow; iv) basal diet with 134 ppm copper as CuMet+glycerol. The composition of the basal diet is provided in Table 1. The diet was provided in mash form and was formulated in accordance with the NRC (1998) recommendations for all nutrients. The energy concentration of liquid glycerol supplemented in this experiment was analyzed of 4,298 kcal/kg of DE and 4,126 kcal/kg of ME. Feed and water were provided *ad libitum* throughout the entirety of the experimental period. The pigs were housed in an environmentally-controlled room with an average temperature of 26°C. The lights remained on from 0800 to 2000 each day.

Sampling and measurements

Body weight and feed intake were measured on d 0, 14, and 35, in order to determine the ADG, ADFI, and G/F values. Chromic oxide (Cr_2O_3) was added (0.2%) as an indigestible marker from days 7 to 14 and days 28 to 35. The fecal samples were collected from at least two pigs per pen via rectal massage, then pooled within the pens. All of the fecal samples, together with the feed samples, were stored in a refrigerator at -20°C until further analysis was conducted. The concentrations of dry matter (DM), nitrogen (N), and energy in the feed and feces were analyzed in accordance with AOAC (1995) procedures. Chromium levels were determined via UV absorption spectrophotometry (UV-1201, Shimadzu, Japan). The N levels were determined using a Kjeltec 2300 Analyzer (Foss Tecator AB, Hoeganaes, Sweden). The gross energy content was determined using an adiabatic bomb calorimeter (Model 1281, Parr, Moline, IL). The DM, N and energy digestibilities were calculated via an indirect method.

Fresh feces and urine samples were randomly collected from at least two pigs in each pen every afternoon between days 33 and 35. The samples were collected at 2-h intervals in containers with sealed lids and were immediately stored at 4°C for the duration of the period. After the collection period, fresh feces and urine were mixed well for each pen. For analysis, feces-urine samples (100 g+500 ml) from each pen were stored in two 2.6-L plastic boxes in duplicate. The plastic boxes were carefully sealed. Each box was equipped with a small hole in the middle of one side wall, which was sealed with adhesive plaster. The samples were allowed to ferment for a period of 30 days at room temperature (25°C). After the fermentation period, a Gastec (Model GV-100) gas sampling pump was utilized for gas detection (Gastec Corp., Gastec detector tube No. 3L and 3La for NH_3; No. 4LL and 4LK for H_2S; No. 70 and 70L for RSH, Gastec Corp, detector tube, Japan). Prior to measurement, the slurry samples were shaken manually for approximately 30 s in order to disrupt any crust formation on the surface of the slurry sample and to homogenize them. The adhesive plaster was then punctured and 100 ml of headspace air was sampled approximately 2.0 cm above the slurry surface.

In order to analyze the fecal copper concentration, the

Table 1. Formula and chemical compositions of diets (as-fed basis)

Ingredients (%)	$CuSO_4$		CuMet	
	Tallow	Glycerol	Tallow	Glycerol
Ground corn	64.20	64.08	64.20	64.08
Soybean meal	23.00	23.00	23.00	23.00
Rice bran	5.00	5.00	5.00	5.00
Corn gluten meal	1.81	1.93	1.81	1.93
Tallow	3.50	-	3.50	-
Glycerol	-	3.50	-	3.50
Dicalcium phosphate	0.88	0.88	0.88	0.88
L-lysine (90%)	0.14	0.14	0.14	0.14
Limestone	0.82	0.82	0.82	0.82
Salt	0.15	0.15	0.15	0.15
Vitamin premix[1]	0.25	0.25	0.25	0.25
Mineral premix[2, 3]	0.25	0.25	0.25	0.25
Chemical composition[4]				
ME (kcal/kg)	3,354	3,215	3,364	3,067
Crude protein (%)	17.89	16.42	17.68	16.16
Lysine (%)	1.32	1.19	1.44	1.26
Calcium (%)	0.70	0.70	0.70	0.70
Phosphorus (%)	0.59	0.59	0.59	0.59

[1] Provided per kg of complete diet: 4,000 IU of vitamin A; 800 IU of vitamin D_3; 17 IU of vitamin E; 2 mg of vitamin K; 4 mg of vitamin B_2; 1 mg of vitamin B_6; 16 μg of vitamin B_{12}; 11 mg of pantothenic acid; 20 mg of niacin and 0.02 mg of biotin.
[2] Provided per kg of complete diet: 175 mg of Fe; 89 mg of Mn; 0.3 mg of I; 0.5 mg of Co and 0.4 mg of Se.
[3] $CuSO_4$ was provided 134 ppm; CuMet was provided 134 ppm. [4] Analyzed values.

dried fecal samples were digested with a microwave digestion system (Model MDS-81D; CEM Corp., Matthews, NC), using a modified version of the procedure previously described by Ward et al. (1996). A representative samples of 0.5-g dried feces were placed in Teflon vessels (CEM Corp. Matthews, NC) with 10 ml of trace metal-grade nitric acid (Fisher Scientific, Pittsburgh, PA) and then allowed to digest at room temperature (23°C) for 30 min. The vessels were sealed and placed in a microwave digester for 5 min at 50% power, followed by 15 min at 70% power, and a final 10 min at 0% power. The vessels were vented, and 2 ml of 30% hydrogen peroxide (Sigma-Aldrich, St. Louis, MO) was added to each vessel. The open vessels were again placed in the microwave digester for 3 min at 50% power and cooled for 2 min at 0% power. The vessels were removed, the samples were transferred to 25-ml volumetric flasks and allowed to cool, and the samples were then brought to the final desired volume with deionized water. The copper concentrations were determined via flame atomic absorption spectrophotometry.

The pigs were monitored for clinical signs of diarrhea and a scoring system was applied. A fecal scoring system was applied for clinical diarrhea evaluation. Feces scoring began on d 0 on the experimental diets and continued until d 35. Scores were assessed daily for individual pens and the average fecal score value per pen was recorded. The following system was used to score the feces: 1 = hard feces, 2 = slightly soft feces in pen, 3 = soft, partially formed feces, 4 = loose, semi-liquid feces, and 5 = watery, mucous-like feces.

Statistical analyses

Data were analyzed as a 2×2 factorial using the GLM procedure of the SAS software package (1996). For the comparison of copper and energy sources, the pen was adopted as the experimental unit, and the model included the effects of copper source, energy source, copper source×energy source interactions. When a significant interaction was observed, the mean values among dietary treatments were separated using the LSD option. The level of significance was established at $p<0.05$.

RESULTS AND DISCUSSION

Growth performance

Growth performance was shown in Table 2. The ADG and ADFI were affected neither by the Cu source or energy source throughout the entirety of the trial period. However, significant interaction ($p = 0.04$) of copper×energy were detected in the G/F ratio during weeks 2-5.

Results similar to those of the present study were previously obtained by Veum et al. (2004), who determined that Cu-proteinate (200 ppm) and $CuSO_4$ (250 ppm)

Table 2. The effect of different copper and energy sources on growth performance in growing pigs[1]

Items	Cu	$CuSO_4$		CuMet		SE[2]	p-value		
	Energy	Tallow	Glycerol	Tallow	Glycerol		Cu	Energy	Cu×Energy
0-2 wk									
ADG (kg)		0.586	0.530	0.588	0.561	0.019	0.62	0.21	0.67
ADFI (kg)		1.207	1.173	1.188	1.223	0.016	0.66	0.98	0.36
Gain/feed		0.486	0.452	0.495	0.457	0.016	0.78	0.16	0.95
2-5 wk									
ADG (kg)		0.447	0.522	0.493	0.403	0.038	0.32	0.84	0.32
ADFI (kg)		1.282	1.243	1.368	1.234	0.044	0.45	0.10	0.36
Gain/feed		0.348^{b}	0.421^{a}	0.362^{ab}	0.328^{b}	0.024	0.13	0.42	0.04
Overall (0-5 wk)									
ADG (kg)		0.503	0.525	0.531	0.466	0.028	0.55	0.41	0.10
ADFI (kg)		1.251	1.215	1.296	1.230	0.030	0.41	0.16	0.68
Gain/feed		0.402	0.433	0.410	0.379	0.018	0.18	0.97	0.07

[1] Cu was supplemented at 134 ppm in $CuSO_4$ or CuMet; energy source was supplemented at 3.50% in tallow or glycerol.
[2] Pooled standard error. [a, b] Means in the same row with difference superscripts differ (p<0.05).

supplementation in the diet of weaning pigs had no significant effects on growth performance over a 28-day experimental period. Also, in accordance with the results of Stansbury et al. (1990), no significant differences in ADG, ADFI, or G/F values were observed when the pigs were fed on 125 ppm $CuSO_4$, inorganic chelated copper, and organic chelated copper during the growing phase (18-64 kg). However, previous research (Zhou et al., 1994) has shown that the effects of copper source (copper sulfate and copper lysine; both 200 ppm) over a 24-day experiment with weanling pigs was significant with regard to ADG and ADFI values. Veum et al. (1995) also reported that replacing a portion of the inorganic trace minerals with proteinate forms improved feed efficiency in nursery pigs. However, during the growing and gilt-developer phases, pig performance was shown to be similar in pigs fed on chelated mineral treatment and those fed on reduced inorganic treatment. In the present study, the detection of a copper×energy interaction (p = 0.04) indicated that the energy source affected the G/F among copper source during weeks 2-5. On G/F, during weeks 2-5, a 4.0% increase in tallow treatment was noted when CuMet was compared with $CuSO_4$ and a 22.1% decrease was also detected in glycerol treatment when CuMet was compared with $CuSO_4$. No similar results have been reported previously. A trend (p = 0.10) toward declining ADFI caused by glycerol was observed, and this may have affected the G/F in weeks 2-5.

Nutrient digestibility

Table 3 shows the effects of different dietary copper and energy sources on digestibilities of DM, nitrogen and energy at the end of the experiment. No significant effects were observed on DM digestibility. The CuMet treatments evidenced higher N digestibility than was seen with the $CuSO_4$ treatments (p = 0.01). The cooper×energy interaction was observed on both N (p = 0.03) and energy digestibility (p<0.01). The energy source had no effect on apparent DM, nitrogen, or energy digestibility.

In the current study, the methionine released from CuMet may evidence a similar effect on the improvement of N digestibility. Dove (1995) researched different levels of fat supplementation in the diets of weanling pigs. No significant differences between 0 and 5% fat addition affected N digestibility. Moreover, Cera et al. (1989) reported that nitrogen digestibility was not affected between postweanling pigs fed on tallow (8%) and vegetable oil (8%) during a 4-wk experiment. However, Cera et al. (1989) noted that energy digestibility was reduced (5.0%) in tallow as compared with what was observed with vegetable oil treatments (namely, coconut and corn oil). In accordance with the present experiment, tallow reduced (5.0%) energy digestibility as compared with glycerol when pigs were fed on a diet supplemented with $CuSO_4$. However, energy digestibility was higher (5.3%) in the tallow treatment group than in the glycerol treatment group when CuMet

Table 3. The effect of different copper and energy sources on nutrient digestibility in growing pigs[1]

Items (%)	Cu	$CuSO_4$		CuMet		SE[2]	p-value		
	Energy	Tallow	Glycerol	Tallow	Glycerol		Cu	Energy	Cu×Energy
Dry matter		81.90	81.92	81.81	81.92	0.32	0.87	0.83	0.88
Nitrogen		80.88^{ab}	79.54^{b}	81.16^{ab}	81.93^{a}	0.57	0.01	0.52	0.03
Energy		77.91^{b}	82.04^{a}	81.64^{a}	77.54^{b}	0.63	0.51	0.98	<0.01

[1] Cu was supplemented at 134 ppm in $CuSO_4$ or CuMet; energy source was supplemented at 3.50% in tallow or glycerol.
[2] Pooled standard error. [a, b] Means in the same row with difference superscripts differ (p<0.05).

Table 4. The effect of different copper and energy sources on fecal noxious gas emission compounds in growing pigs[1]

Items (ppm)	Cu	$CuSO_4$		CuMet		SE[2]	p-value		
	Energy	Tallow	Glycerol	Tallow	Glycerol		Cu	Energy	Cu×Energy
NH_3		6.33	1.27	0.00	0.00	2.00	0.01	0.27	0.74
RSH		9.33	4.67	9.33	3.33	2.03	0.58	0.04	0.60
H_2S		11.67	2.00	14.00	0.00	5.55	0.50	0.02	0.31

[1] Cu was supplemented at 134 ppm in $CuSO_4$ or CuMet; energy source was supplemented at 3.50% in tallow or glycerol.
[2] Pooled standard error.

was added to the diet. This copper×energy interaction remains to be clearly elucidated, and thus warrants further study.

Fecal noxious gas emission compounds

Table 4 shows the effects of copper and energy sources on gas emission compounds. NH_3 emission was significantly lower when CuMet was used as a source than when $CuSO_4$ was used (p = 0.01). RSH and H_2S emissions were higher in the tallow treatment group than in the glycerol treatment group (p = 0.04; p = 0.02, respectively). No copper×energy interaction was detected in the context of NH_3, H_2S and RSH emissions.

The emission of NH_3 from pig manure is influenced principally by the manure ammonium concentration (Canh et al., 1998). The ammonium nitrogen content of pig feces was reduced when crystalline amino acids were incorporated into the diet (Aarnink et al., 1998). The methionine contained in CuMet can be released when copper is absorbed in its free ionic form. This explains the reduction of NH_3 emission observed in conjunction with the CuMet treatments.

Variations in sulfuric odorous compound concentrations in animal feces were attributed principally to differences in the sulfur composition of the diets fed to the pigs and the metabolism of sulfur-containing amino acids (methionine, cystine, and cysteine), which generate sulfuric odorous compounds including H_2S and RSH (Kiene and Hines, 1995). The H_2S and RSH are important sulfur-containing volatile constituents (Spoelstra, 1980). In this study, we demonstrated that RSH and H_2S emissions were not influenced by sulfate or methionine. However, the mechanism by which the energy source affects the sulfuric odorous compound concentrations in animal feces remains to be clearly elucidated. More research will be required in order to determine the differences between RSH and H_2S emission levels between tallow and glycerol.

Diarrhea appearance

Table 5 shows the effects of copper (inorganic and organic) and energy (tallow and glycerol) sources on the appearance of diarrhea in nursery pigs. No significant effects of copper or energy source or copper×energy interaction were observed throughout the entirety of the experimental period.

Previous studies (Hill et al., 2000; Hedemann et al., 2006) concerning copper supplementation in pigs suggested that the addition of copper with ZnO could reduce both the incidence and severity of diarrhea. The antibiotic-like effects of dietary copper on the microflora of the intestinal tract is considered to be a principal factor in the control of diarrhea in young pigs (Braude, 1967; Cromwell, 2001). In the current study, organic and inorganic copper were shown to exert identical effects on diarrhea. This supports the results of a study by Veum et al. (2004), Armstrong et al. (2004), and Creech et al. (2004), who determined that pig feed supplements of Zn, Cu, Fe, and Mn from chelated metal proteinates evidenced equal or higher availability than similar concentrations of trace minerals solely in their inorganic sulfate forms.

Fecal Cu concentration

At the end of weeks 2 and 5, the fecal copper concentrations were significantly lower in the CuMet

Table 5. The effect of different copper and energy sources on diarrhea appearance in growing pigs[1]

Items	Cu	$CuSO_4$		CuMet		SE[2]	p-value		
	Energy	Tallow	Glycerol	Tallow	Glycerol		Cu	Energy	Cu×Energyt
No. of pigs		24	24	24	24				
No. of diarrhea pigs[3]									
0-2 weeks (Score)		2.9	2.8	2.2	2.6	0.91	0.58	0.92	0.13
2-5 weeks (Score)		2.7	2.1	2.1	2.3	0.75	0.78	0.41	0.50
Overall (Score)		1.5	2.1	2.3	1.2	0.79	0.43	0.41	0.98

[1] Cu was supplemented at 134 ppm in $CuSO_4$ or CuMet; energy source was supplemented at 3.50% in tallow or glycerol.
[2] Pooled standard error.
[3] 1 = hard feces, 2 = slightly soft feces in pen, 3 = soft, partially formed feces, 4 = loose, semi-liquid feces, and 5 = watery, mucous-like feces.

Table 6. The effect of different copper and energy sources on fecal Cu concentration in growing pigs[1]

Items (ppm)	Cu	$CuSO_4$		CuMet		SE[2]	P-value		
	Energy	Tallow	Glycerol	Tallow	Glycerol		Cu	Energy	Cu×Energy
Cu concentration in diet									
2 wk		116.98	117.68	118.55	118.09	0.68	-	-	-
5 wk		117.54	114.81	114.05	117.74	1.46	-	-	-
Cu concentration in feces									
2 wk		68.41	71.81	69.74	62.89	1.99	0.02	0.43	0.62
5 wk		66.93	57.76	59.43	55.77	1.10	0.02	0.08	0.55

[1] Cu was supplemented at 134 ppm in $CuSO_4$ or CuMet; energy source was supplemented at 3.50% in tallow or glycerol.
[2] Pooled standard error.

treatment group than in the $CuSO_4$ treatment group (5.7%, p = 0.03; 8.2%, p = 0.02, respectively). The primary effects of energy source or copper×energy interactions were not significant.

In the current study, CuMet treatments evidenced an effect on the reduction of fecal copper concentrations as compared with the $CuSO_4$ treatments. This result was in accordance with the results reported by Creech et al. (2004), who reported that pigs fed chelated metal proteinates evidenced lower (p<0.01) fecal copper concentrations than were observed in pigs fed on an inorganic metal diet during the growth phase. In some studies, organically bound copper has been added to swine diets, revealing that more copper can be absorbed and retained more efficiently, and less can be excreted than when pigs are fed with copper in the form of $CuSO_4$ (Apgar et al., 1995; Veum et al., 2004). In accordance with the results of previous studies (Fouad, 1976), the absorption of chelated metals is higher than that of metals in their inorganic form, and the retention of organic metals is also higher than that of inorganic metals, which is the principal factor in the decline in fecal copper concentrations in organic copper supplementation treatments, as seen in the current study.

Reducing copper levels in swine waste is crucial, because the accumulation of these minerals in soil can result in plant toxicity (Tucker, 1997; Matsui and Yano, 1998), thus potentially influencing the sustainability of large swine operations.

CONCLUSION

The data in this study show that organic chelated copper dietary supplementation results in lower levels of fecal copper excretion than does inorganic copper. Moreover, energy sources interact with copper sources, and influence N and energy digestibility. Glycerol can also be used to replace tallow in the diet.

ACKNOWLEDGMENT

The author would like to acknowledge the financial assistance provided by the Agricultural R&D Product Center, Korea.

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