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ANIMAL

Weaning pig performance can be enhanced by replacing dietary inorganic copper and zinc with glycine or methionine-chelated copper and zinc

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Abstract

A total of 180 21-day-old weaning pigs ([Yorkshire × Landrace] × Duroc) with an initial body weight of 6.44 ± 0.01 kg were randomly assigned to 9 treatments for evaluating the effects of replacing dietary inorganic copper (Cu) and zinc (Zn) with glycine (Gly) or methionine (Met)-chelated Cu and Zn on growth performance and nutrient digestibility. The experimental period was 35 days. There were four replicated pens per treatment, with five pigs (three males and two females) per pen. Dietary treatments consisted of a basal diet (CON), in which the sources of Cu and Zn were in inorganic form. The inorganic Cu and Zn in the basal diet were replaced by glycine-chelated (GC) and methionine-chelated (MC) Cu and Zn by 30, 50, 70, or 100% to form the GC1, GC2, GC3, GC4, or MC1, MC2, MC3, MC4 groups. The 100% replacement of dietary inorganic Cu and Zn with GC or MC increased ($p < 0.05$) average daily gain, average daily feed intake, and gain-to-feed ratio. The complete replacement of dietary inorganic Cu and Zn with GC or MC led to enhanced ($p < 0.05$) digestibility of dry matter, nitrogen, Cu and Zn. Thus, the replacement of inorganic Cu and Zn with GC or MC can improve the growth efficiency and nutrient utilization of weaning pigs.

Keywords: amino acid chelated organic mineral, growth efficiency, inorganic mineral, nutrient utilization, weaning pig

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Introduction

Copper (Cu) and zinc (Zn) are vital minerals for maintaining the growth and normal metabolism of animals. The deficiency of Cu could damage the growth promotion factor and impair the growth and antioxidant capacity of animals (Ding et al., 2021). The deficiency of Zn will lead to diarrhea, anorexia, and/or growth retardation in animals (Górniak et al., 2018; Chabaev et al., 2020). Under farm conditions, it is essential to provide an adequate amount of minerals in the diets of pigs to ensure healthy growth (Yue et al., 2017). In general, the supplemented minerals in the diet of livestock are used in inorganic form. However, due to the dissociation of inorganic minerals in the upper intestine, antagonism and interactions may occur among trace minerals, which in turn interfere with the absorption of minerals in animals (Upadhaya and Kim, 2020). To this end, animal nutritionists are considering replacing dietary inorganic minerals with amino acid-chelated minerals which have higher digestive tract absorbability, to enhance the absorption of the minerals. The antagonism and interactions between trace minerals are also prevented by amino acid-chelated minerals, because they have more absorbing ability than inorganic minerals (Nitrayova et al., 2012; Liu et al., 2016).

Methicillin (Met)-Zn's robust molecular structure inhibits combination with harmful substances like phytic acids to generate insoluble substances and impairment of gastric acid production. As a result, metal particles penetrate smoothly the site of absorption, ensuring very effective absorption (Wan et al., 2018). Met-Zn and other trace component amino acid chelates typically exhibit greater biological impact than the individual trace elements and amino acids. It also has a variety of unique physiological effects, including increased protein and vitamin utilization rates, involvement in intracellular redox responses, and modulation of enzyme functions in living things (Case and Carlson, 2002).

Glycine or methionine-chelated Cu or Zn can effectively improve the growth efficiency, nutrient utilization, and health status of pigs and decrease gas emissions to reduce environmental pollution compared with inorganic Cu or Zn (Li et al., 2011; Nitrayova et al., 2012; Barszcz et al., 2019). Zn methionine chelate (MC) showed greater biological activity, enhanced piglet growth efficiency, and modified immune functions, in comparison to conventional Zn supplements (Chen et al., 2019). Moreover, chelated Zn instead of therapeutic Zn oxide (ZnO) may improve growth, control fecal bacteria levels, lower fecal scores, and lessen environmental pollution, according to Biswas et al. (2024). Due to the benefits in growth performance and carcass features, the chelated Cu can be replaced by high $CuSO_4$ as a growth stimulant in pigs (Zhao et al., 2014). In comparison to inorganic minerals, Cu chelate was more effective in avoiding nutritional antagonistic interactions and required to achieve higher efficiency in broilers and nursery piglets (Zhao et al., 2009; 2010). Since glycine-chelate Zn is more soluble in the stomach than Zn sulfate ($ZnSO_4$), it is more conducive to absorbing Zn when phytates are presented (Schlegel et al., 2010).

We hypothesized that the replacement of dietary inorganic Cu and Zn with (glycine-chelated) GC or MC could improve the apparent nutrient utilization and growth performance of weaning pigs. Taking inorganic minerals in the form of sulfate as the control group, the effects of substituting dietary inorganic Cu and Zn in the form of sulfate with glycine (Gly) or methionine (Met) chelated Cu and Zn on growth performance and apparent digestibility of nutrients in weaning pigs were aimed to assess.

Materials and Methods

The protocol was reviewed and approved by the Institutional Animal Care and Use Committee at the University of Dankook. All animal procedures were followed by the Animal Care and Use Committee of Dankook University, Cheonan, South Korea (Approval No. DK-2-2013).

Experimental design and diets

In total, 180 21-day-old crossbred weaning piglets ([Yorkshire × Landrace] × Duroc) with 6.44 ± 0.01 kg of preliminary body weight were arbitrarily allotted to 9 treatments, with each treatment consisting of four repetition pens with five pigs (three males and two females) each. During a 35-day feeding trial, the experiment was divided into two phases (phase

1, days 1 - 14; phase 2, days 15 - 35). The feed compositions were the same in dietary treatments (as-fed basis) except for the source of dietary Cu and Zn. The dietary treatments included a basal diet (CON) with inorganic supplements of Cu and Zn. The chelated Gly or Met minerals were substituted with corn. The used ZnO source was a feed-grade source. The inorganic and organic minerals were provided by the Daehan Chemtech Co., Ltd. (Korea). The complexity of the diets was modified with phases to fulfill the National Research Council's (NRC, 2012) suggested nutritional requirement and to satisfy changes in the digestive capabilities of the weaning pigs (Table 1 and 2).

Table 1. Experimental diet composition as fed-basis during days 1 - 14.

Item	CON	GC1	GC2	GC3	GC4	MC1	MC2	MC3	MC4
Corn	34.8656	34.8584	34.8536	34.8489	34.8416	34.8298	34.8060	34.7821	34.7463
Corn (extra pure)	15.00	15.00	15.00	15.00	15.00	15.00	15.00	15.00	15.00
Lactose	10.00	10.00	10.00	10.00	10.00	10.00	10.00	10.00	10.00
Dehulled soybean meal	13.50	13.50	13.50	13.50	13.50	13.50	13.50	13.50	13.50
Concentrated soybean meal	10.00	10.00	10.00	10.00	10.00	10.00	10.00	10.00	10.00
Plasma protein	6.00	6.00	6.00	6.00	6.00	6.00	6.00	6.00	6.00
Whey protein	5.00	5.00	5.00	5.00	5.00	5.00	5.00	5.00	5.00
Soy oil	2.20	2.20	2.20	2.20	2.20	2.20	2.20	2.20	2.20
Limestone	1.20	1.20	1.20	1.20	1.20	1.20	1.20	1.20	1.20
Monocalcium phosphate	1.23	1.23	1.23	1.23	1.23	1.23	1.23	1.23	1.23
DL-Methionine	0.15	0.15	0.15	0.15	0.15	0.15	0.15	0.15	0.15
L-Lysine-HCl	0.06	0.06	0.06	0.06	0.06	0.06	0.06	0.06	0.06
Choline chloride	0.10	0.10	0.10	0.10	0.10	0.10	0.10	0.10	0.10
Zinc oxide	0.032	0.032	0.032	0.032	0.032	0.032	0.032	0.032	0.032
Vitamin mix[y]	0.10	0.10	0.10	0.10	0.10	0.10	0.10	0.10	0.10
Mineral mix[z]	0.10	0.10	0.10	0.10	0.10	0.10	0.10	0.10	0.10
$CuSO_4$	0.0338	0.0237	0.0169	0.0101	-	0.0237	0.0169	0.0101	-
$ZnSO_4$	0.0286	0.0200	0.0143	0.0086	-	0.0200	0.0143	0.0086	-
Gly-Cu	-	0.0144	0.0240	0.0335	0.0479	-	-	-	-
Gly-Zn	-	0.0115	0.0192	0.0269	0.0385	-	-	-	-
Met-Cu	-	-	-	-	-	0.0345	0.0575	0.0805	0.1150
Met-Zn	-	-	-	-	-	0.0200	0.0333	0.0467	0.0667
Salt	0.40	0.40	0.40	0.40	0.40	0.40	0.40	0.40	0.40
Calculated value (%)									
Metabolizable energy (MJ/kg)	14.37	14.37	14.37	14.37	14.37	14.37	14.37	14.37	14.37
Crude protein	20.76	20.76	20.76	20.76	20.76	20.76	20.76	20.76	20.76
Crude fat	4.31	4.31	4.31	4.31	4.31	4.31	4.31	4.31	4.31
Crude fiber	1.85	1.85	1.85	1.85	1.85	1.85	1.85	1.85	1.85
Crude ash	6.38	6.38	6.38	6.38	6.38	6.38	6.38	6.38	6.38
Calcium	0.82	0.82	0.82	0.82	0.82	0.82	0.82	0.82	0.82
Total phosphorus	0.65	0.65	0.65	0.65	0.65	0.65	0.65	0.65	0.65
Lysine	1.35	1.35	1.35	1.35	1.35	1.35	1.35	1.35	1.35
Methionine	0.39	0.39	0.39	0.39	0.39	0.39	0.39	0.39	0.39
Copper	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01
Zinc	0.03	0.03	0.03	0.03	0.03	0.03	0.03	0.03	0.03

CON, control; GC, glycine-chelate; MC, methionine-chelate; HCL, hydrochloride; $CuSO_4$, copper sulfate; $ZnSO_4$, zinc sulfate; Gly-Cu, glycine-chelated copper; Gly-Zn, glycine-chelated zinc; Met-Cu, methionine-chelated copper; Met-Zn, methionine-chelated zinc.

[y] Provided per kg of complete diet: vitamin A, 11,025 IU; vitamin D_3, 1,103 IU; vitamin E, 44 IU; vitamin K, 4.4 mg; riboflavin, 8.3 mg; niacin, 50 mg; thiamine, 4 mg; D-pantothenic, 29 mg; choline, 166 mg; vitamin B_{12}, 33 µg.

[z] provided per kg of complete diet: Fe, 80 mg as ferrous sulfate; Mn, 8 mg as manganese oxide; I, 0.28 mg as potassium iodide; Se, 0.15 mg as sodium selenite.

Table 2. Experimental diet composition as fed-basis during days 15 - 35.

Item	CON	GC1	GC2	GC3	GC4	MC1	MC2	MC3	MC4
Corn	60.8856	60.8784	60.8736	60.8689	60.8616	60.8498	60.8260	60.8021	60.7663
Corn (extra pure)	5.00	5.00	5.00	5.00	5.00	5.00	5.00	5.00	5.00
Lactose	3.00	3.00	3.00	3.00	3.00	3.00	3.00	3.00	3.00
Dehulled soybean meal	13.00	13.00	13.00	13.00	13.00	13.00	13.00	13.00	13.00
Concentrated soybean meal	10.00	10.00	10.00	10.00	10.00	10.00	10.00	10.00	10.00
Plasma protein	3.00	3.00	3.00	3.00	3.00	3.00	3.00	3.00	3.00
Soy oil	2.00	2.00	2.00	2.00	2.00	2.00	2.00	2.00	2.00
Limestone	0.99	0.99	0.99	0.99	0.99	0.99	0.99	0.99	0.99
Monocalcium phosphate	1.18	1.18	1.18	1.18	1.18	1.18	1.18	1.18	1.18
DL-Methionine	0.04	0.04	0.04	0.04	0.04	0.04	0.04	0.04	0.04
L-Lysine-HCL	0.11	0.11	0.11	0.11	0.11	0.11	0.11	0.11	0.11
Choline chloride	0.10	0.10	0.10	0.10	0.10	0.10	0.10	0.10	0.10
Zinc oxide	0.032	0.032	0.032	0.032	0.032	0.032	0.032	0.032	0.032
Vitamin mix[y]	0.10	0.10	0.10	0.10	0.10	0.10	0.10	0.10	0.10
Mineral mix[z]	0.10	0.10	0.10	0.10	0.10	0.10	0.10	0.10	0.10
$CuSO_4$	0.0338	0.0237	0.0169	0.0101	-	0.0237	0.0169	0.0101	-
$ZnSO_4$	0.0286	0.0200	0.0143	0.0086	-	0.0200	0.0143	0.0086	-
Gly-Cu	-	0.0144	0.0240	0.0335	0.0479	-	-	-	-
Gly-Zn	-	0.0115	0.0192	0.0269	0.0385	-	-	-	-
Met-Cu	-	-	-	-	-	0.0345	0.0575	0.0805	0.1150
Met-Zn	-	-	-	-	-	0.0200	0.0333	0.0467	0.0667
Salt	0.40	0.40	0.40	0.40	0.40	0.40	0.40	0.40	0.40
Calculated value (%)									
Metabolizable energy (MJ/kg)	14.18	14.18	14.18	14.18	14.18	14.18	14.18	14.18	14.18
Crude protein	18.78	18.78	18.78	18.78	18.78	18.78	18.78	18.78	18.78
Crude fat	4.52	4.52	4.52	4.52	4.52	4.52	4.52	4.52	4.52
Crude fiber	2.16	2.16	2.16	2.16	2.16	2.16	2.16	2.16	2.16
Crude ash	5.64	5.64	5.64	5.64	5.64	5.64	5.64	5.64	5.64
Calcium	0.70	0.70	0.70	0.70	0.70	0.70	0.70	0.70	0.70
Total phosphorus	0.60	0.60	0.60	0.60	0.60	0.60	0.60	0.60	0.60
Lysine	1.15	1.15	1.15	1.15	1.15	1.15	1.15	1.15	1.15
Methionine	0.30	0.30	0.30	0.30	0.30	0.30	0.30	0.30	0.30
Copper	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01
Zinc	0.03	0.03	0.03	0.03	0.03	0.03	0.03	0.03	0.03

CON, control; GC, glycine-chelate; MC, methionine-chelate; HCL, hydrochloride; $CuSO_4$, copper sulfate; $ZnSO_4$, zinc sulfate; Gly-Cu, glycine-chelated copper; Gly-Zn, glycine-chelated zinc; Met-Cu, methionine-chelated copper; Met-Zn, methionine-chelated zinc.

[y] Provided per kg of complete diet: vitamin A, 11,025 IU; vitamin D_3, 1,103 IU; vitamin E, 44 IU; vitamin K, 4.4 mg; riboflavin, 8.3 mg; niacin, 50 mg; thiamine, 4 mg; D-pantothenic, 29 mg; choline, 166 mg; vitamin B_{12}, 33 µg.

[z] Provided per kg of complete diet: Fe, 80 mg as ferrous sulfate; Mn, 8 mg as manganese oxide; I, 0.28 mg as potassium iodide; Se, 0.15 mg as sodium selenite.

Animals and housing

The piglets were kept in a temperature-controlled environment that was kept at 30℃ for the first week and 25℃ for the remainder of the trial with 60% humidity. The floor of the barn was constructed with a slatted plastic base. A single-sided feeder and nipple drinker were equipped to ensure the animals had convenient access to feed and water.

Sample collection and measurements

Individual body weight was measured on days 1, 14, and 35 to calculate the average daily gain (ADG). Pen-based feed intake was checked daily to calculate the average daily feed intake (ADFI). The gain-to-feed (G/F) ratio was calculated using ADG and ADFI values.

During days 28 to 35, 0.20% chromium oxide was added to the diet to determine the apparent total tract digestibility (ATTD) of dry matter (DM), nitrogen, Cu, and Zn, and apparent retention of energy. The representative feed samples were collected from each dietary treatment after being mixed. On day 35, two pigs were randomly selected from each pen to take fecal samples via the rectal massage method. For each collected fecal and diet sample, 10% hydrochloric acid was added for the fixation of nitrogen. Then, feed and fecal samples were dried using an oven at 70℃ for 72 hours, and later they were ground to pass through a 1-mm sieve and collected. The DM in feed and feces was assessed according to the Association of Official Analytical Chemists (AOAC, 2000), involving over-drying at 135℃ for 2 hours (method 930.15). Crude protein was measured using the Kjeldahl method (nitrogen × 6.25; method 968.06). The Cu and Zn concentrations were performed using AOAC (2000) method 985.01. Gross energy contents were assessed by a bomb calorimeter (Parr 6100, Parr Instrument Co., USA). In addition, following the procedure established by the AOAC (2005), diet samples were analyzed for crude fiber (method 991.43), calcium (method 984.01), phosphorus (method 965.17), and crude fat (method 954.02). The lysine and Met contents of the diets were measured using an amino acid analyzer (Beckman 6300, Beckman Coulter Inc., USA). Chromium levels were determined via UV absorption spectrophotometry (UV-1201, Shimadzu, Japan). The indirect-ratio methods were used to calculate the ATTD using the formula used by Biswas and Kim (2022).

Statistical analysis

All the data were evaluated using the general linear model method (SAS version 9.4, SAS Institute Inc., USA), and the pen was utilized as the experimental unit in a randomized block design. The treatment groups were differentiated using preplanned contrasts. Contrast comparisons were carried out among treatment means to compare Gly or Met chelated minerals containing diet, and level of 100% for all mineral sources as well. The standard error of means (SEM) was used to depict the variety in the data. Significant differences were examined by $p < 0.05$ and $p < 0.10$ was deliberated as a trend.

Results and Discussion

The complete replacement (100%) of dietary inorganic Cu and Zn increased ADG at days 1 -14 ($p = 0.002$) and 1 - 35 ($p = 0.030$), ADFI at days 1 - 14 ($p = 0.005$), and the gain-to-feed ratio at days 1 - 14 ($p = 0.004$) (Table 3). No significant differences in different level variability ($p > 0.05$) were observed between replacing dietary inorganic Cu and Zn with Met-chelated Cu and Zn. Replacing dietary inorganic minerals in the sulfate form with amino acid-chelated organic minerals has been demonstrated to be beneficial to the performance of pig growth (Zhan et al., 2014). In addition, Chen et al. (2019) noted that replacing dietary inorganic minerals with Met-chelated minerals could increase the ADG of weaning pigs, while ADFI showed no significant difference. Similarly, replacing dietary $ZnSO_4$ with Gly-chelated Zn increased the ADG, ADFI, and G/F ratios of pigs (Barszcz et al., 2019). Likewise, the incorporation of chelated Zn improved ADG and the G/F ratio in growing pigs (Jiao et al., 2020). Conversely, organically bound trace minerals showed no significant differences regarding to the magnitude of improvement in ADG, ADFI, and G/F ratio (Huang et al., 2010; Liu et al., 2016). As stated by Biswas et al. (2024), the dietary inclusion of Zn aspartic acid chelate instead of therapeutic ZnO could improve body weight, ADG, and ADFI. The inorganic Cu and Zn in the form of sulfate had inferior absorbability to Gly or Met chelated Cu and Zn. So the enhancement of the apparent digestibility of Cu and Zn by replacing dietary inorganic Cu and Zn with Gly or Met chelated Cu and Zn in our study was thought to be the cause of the enhancement in growth parameters.

Table 3. Effects of replacing dietary inorganic Cu and Zn with Gly or Met chelated Cu and Zn on growth performance of weaning pigs.

Minerals in organic form (%)	Minerals in inorganic form (%)	ADG (g)			ADFI (g)			Gain to feed ratio		
		Days 1 - 14	Days 15 - 35	Days 1 - 35	Days 1 - 14	Days 15 - 35	Days 1 - 35	Days 1 - 14	Days 15 - 35	Days 1 - 35
-	100	258.93	460.17	379.67	351.15	690.86	554.97	0.74	0.67	0.68
Minerals in Gly-chelated form										
GC1 30	70	276.07	489.71	404.26	368.93	713.81	575.86	0.75	0.69	0.70
GC2 50	50	272.14	496.24	406.60	365.36	720.22	578.27	0.75	0.69	0.70
GC3 70	30	268.93	486.38	399.40	362.86	710.29	571.31	0.74	0.69	0.70
GC4 100	-	281.43	500.57	412.91	372.14	724.91	583.80	0.76	0.69	0.71
Minerals in Met-chelated form										
MC1 30	70	265.00	479.48	393.69	358.93	703.98	565.96	0.74	0.68	0.70
MC2 50	50	259.64	461.90	381.00	351.79	692.81	556.40	0.74	0.67	0.69
MC3 70	30	267.50	482.62	396.57	362.14	705.69	568.27	0.74	0.69	0.70
MC4 100	-	283.57	492.98	409.21	374.86	716.95	580.12	0.76	0.69	0.71
p-value										
Inorganic minerals vs organic minerals		0.184	0.170	0.122	0.292	0.235	0.192	0.321	0.263	0.215
100% of each source		0.184	0.170	0.122	0.292	0.235	0.192	0.321	0.263	0.215
Pooled SEM		5.847	11.935	8.218	7.609	13.706	9.688	0.010	0.010	0.008

Cu, copper; Zn, zinc; Gly, glycine; Met, methionine; ADG, average daily gain; ADFI, average daily feed intake; GC, glycine-chelate; MC, methionine-chelate; SEM, standard error of the mean.

The apparent digestibility of DM ($p = 0.049$), nitrogen ($p = 0.001$), Cu ($p = 0.010$), and Zn ($p = 0.003$) was all enhanced by completely replacing dietary inorganic Cu and Zn with Gly or Met chelated Cu and Zn. However, the replacement of dietary inorganic Cu and Zn with Met-chelated Cu and Zn in various levels of variability did not result ($p > 0.05$) in any appreciable differences (Table 4). Feeding pigs with a Met-chelated Cu-containing diet led to a higher apparent digestibility of nitrogen than those fed with an inorganic mineral-containing diet but had no effect on DM or energy digestibility (Huang et al., 2010). Barszcz et al. (2019) observed a reduction in Zn digestibility with Zn-glycine supplement compared to Zn sulfate. As mentioned by Schlegel et al. (2010), piglets given organic Zn had greater gastrointestinal Zn absorption than those given inorganic Zn. In contrast to our experiment, the dietary administration of chelated Zn had significant impacts on the nutrient digestibility of DM, but no significant effects were found in the nutrient utilization of nitrogen, energy, and Zn (Jiao et al., 2020). The positive effects of replacing dietary inorganic minerals with amino acid-chelated minerals on nutrient digestibility may be related to the improvement of the intestinal environment and the reduction of intestinal stress levels (Wang et al., 2010). According to our study, replacing dietary inorganic Cu and Zn with Gly or Met chelated Cu and Zn can improve the apparent digestibility of nutrients in weaning pigs, which helps improve their performance.

Table 4. Effects of replacing dietary inorganic Cu and Zn with Gly or Met chelated Cu and Zn on apparent nutrient digestibility of weaning pigs.

Minerals in organic form (%)		Minerals in inorganic form (%)	DM	Nitrogen	Energy	Cu	Zn
	-	100	77.87	73.94	78.16	37.70	30.92
Minerals in Gly-chelated form							
GC1	30	70	78.97	75.19	77.80	38.46	31.16
GC2	50	50	79.30	77.81	78.13	40.44	34.23
GC3	70	30	78.24	74.47	77.37	41.05	35.63
GC4	100	-	80.13	77.87	80.02	42.53	36.44
Minerals in Met-chelated form							
MC1	30	70	79.53	75.77	77.43	38.07	31.00
MC2	50	50	79.94	77.03	79.39	40.43	31.72
MC3	70	30	79.52	75.48	77.67	42.38	36.60
MC4	100	-	80.06	74.67	79.00	43.20	36.85
p-value							
Inorganic minerals vs organic minerals			0.225	0.499	0.937	0.702	0.796
100% of each source			0.049	0.001	0.419	0.010	0.003
Pooled SEM			0.748	1.199	0.903	1.847	1.795

Cu, copper; Zn, zinc; Gly, glycine; Met, methionine; DM, dry matter; GC, glycine-chelate; MC, methionine-chelate; SEM, standard error of the mean.

Conclusion

In conclusion, the replacement of dietary inorganic Cu and Zn with Gly or Met chelated Cu and Zn can improve the ADG, ADFI, gain-to-feed ratio, and apparent digestibility of DM, nitrogen, Cu, and Zn in weaning pigs. Thus, replacing dietary inorganic Cu and Zn with Gly or Met chelated Cu and Zn was a suitable strategy for improving the growth performance and nutrient digestibility of weaning pigs.

Conflict of Interests

No potential conflict of interest relevant to this article was reported.

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