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Influence of tetrabasic zinc chloride and copper sulphate on growth performance and some physiological parameters in the digestive tract of weanling piglets*

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ABSTRACT

One hundred and sixty weanling piglets with an initial average body weight (BW) of 7.3 kg were used in a 2×2 factorial experiment to investigate interactions between tetrabasic zinc chloride (TBZC) and copper sulphate ($CuSO_4$) on growth performance. In five piglets per treatment activity of enzymes in pancreas and digesta and short-chain fatty acids (SCFA) concentration in digesta were determined. The piglets were offered the following diets for 28 days: 1. no supplementation of TBZC and $CuSO_4$; 2. supplementation of 250 mg/kg Cu from $CuSO_4$; 3. supplementation of 1500 mg/kg Zn from TBZC; 4. supplementation of 1500 mg/kg Zn from TBZC and 250 mg/kg Cu from $CuSO_4$. Each treatment had five replications (pens) of eight piglets (half castrated males and half females). The results showed that average daily gain (ADG) was improved ($P<0.05$) by the addition of TBZC and (or) $CuSO_4$. The addition of TBZC increased ($P<0.05$) the activity of chymotrypsin in the duodenum contents (3.145 vs 2.095, respectively). TBZC and $CuSO_4$ synergistically reduced ($P<0.05$) pH of the jejunum contents. The addition of TBZC or $CuSO_4$ increased ($P<0.05$) total SCFA concentrations, and the proportion of acetic acid and propionic acid in the colon. TBZC reduced ($P<0.05$) the proportions of isobutyric acid (1.470 vs 1.830, respectively) and isovaleric acid (0.171 vs 0.203, respectively), but $CuSO_4$ increased ($P<0.01$) the proportions of isobutyric acid (1.904 vs 1.396, respectively) in the colon. There was a significant interaction between TBZC and $CuSO_4$ for ADG ($P<0.01$), for trypsin activities in both pancreatic tissue homogenates ($P<0.01$) and duodenal contents ($P<0.05$) and for chymotrypsin activities in both pancreatic tissue homogenate

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($P<0.05$) and jejunum contents ($P<0.01$), and for total SCFA concentrations in the colon. These data indicated that the growth-promotional mechanisms of TBZC and $CuSO_4$ might be different.

KEY WORDS: tetrabasic zinc chloride, copper sulphate, enzymes, SCFA, growth performance, piglets

INTRODUCTION

Feeding high level of Zn to weanling piglet could decrease the incidence of non-specific post-weanling scours and improve growth performance (Case and Carlson, 2002; Zhang and Guo, 2007). However, the mechanism of this action is obscure. Our previous experiment indicated that feeding pharmacological tetrabasic Zn chloride (TBZC) improved growth performance of weanling piglets, and that TBZC is a better Zn source compared with ZnO for weanling piglet diets to enhance growth performance at lower dosage (Zhang and Guo, 2007).

High dietary concentrations of copper sulphate ($CuSO_4$) have long been recognized for its growth-promoting effects in diets for weanling piglets (Bunch et al., 1961; Radecki et al., 1992). Feeding 250 mg/kg Cu from $CuSO_4$ improved growth performance of weanling piglets (Armstrong et al., 2004). Previous research indicated that there was an interaction between high level of zinc oxide (ZnO) and $CuSO_4$, but additive responses to growth-promoting were not observed (Smith et al., 1997; Hill et al., 2000). However, recently research reported that organic copper and ZnO had additive benefits effects on piglet's performance (Pettigrew and Perez-Mendoza, 2007). The additive effects of TBZC and $CuSO_4$ had not been tested.

Weanling of piglets is commonly followed by a depression in growth rate and diarrhoea during first one and (or) two weeks after weaning. Decrease in the activity of digestive enzymes in the pancreatic tissue after weaning has been reported which has been suggested to give a predisposition to the poor growth performance (Hedemann and Jensen, 2004). The pH in the intestine is an important factor influencing the activity of digestive enzymes. Changes in pH outside normal ranges can result in decreasing digestive ability and eventually reducing growth performance. In addition, intestinal pH is also important to keep animal intestinal health by maintaining the balance between nonpathogenic and pathogenic microorganisms. The quantity of short-chain fatty acid (SCFA) produced in the intestine depends on the types and numbers of different bacterial population in the gut (MacFarlane and MacFarlane, 2003). Cu and Zn had been recognized as having antibacterial activity (Dupont et al., 1994). Measurement of intestinal responses, such as pH and SCFA, had been used to determine gut health in piglet. It is speculated that supplementing piglet diets with TBZC and $CuSO_4$ may make an intestinal environment unsuitable for pathogenic bacterial growth.

One objective of this experiment was to evaluate the interactive effects of high concentrations of dietary Zn as TBZC, and Cu as $CuSO_4$ on growth performance of weanling piglets. The other objective of this experiment was to further investigate the effect of a growth-stimulating level of TBZC and $CuSO_4$ on the activity of digestive enzymes, intestinal pH and SCFA concentration of weanling piglets.

MATERIAL AND METHODS

Animals and diets

The experiment was designed as a 2×2 factorial. One hundred and sixty piglets (Landrace × Large White), weaned at 27±1 days of age with an average initial weight of 7.3 kg, were allotted to pens on the basis of similar body weight (BW), ancestry and gender. Each treatment had five replications (pens) of eight piglets (half castrated males and half females). Piglets were housed on hard plastic slotted floors with self-feeders, and automatic stainless nipple waterers. Feed and water were available *ad libitum*. The basal diets with approximately 138 mg Zn/kg and 16 mg Cu/kg were formulated to meet or exceed nutrient requirements recommended by NRC (1998). Compositions of the diets and nutrient levels for 1-14 d or 15-28 d are shown in Table 1. The four experimental diets were as follows: 1. no supplementation of TBZC and $CuSO_4$; 2. supplementation of 250 mg/kg Cu from $CuSO_4$; 3. supplementation of 1500 mg/kg Zn from TBZC; 4. supplementation of 1500 mg/kg Zn from TBZC and 250 mg/kg Cu from $CuSO_4$. The tested levels for Zn and Cu are outside of the European Union-upper levels (Zn: 150 mg/kg, Cu: 170 mg/kg up to 12 weeks of age). The TBZC [$Zn_5(OH)_8Cl_2 \cdot H_2O$] contained 58 Zn%. Both the TBZC and (or) $CuSO_4$ replaced wheat bran in the diet. The experiment lasted 28 days.

Piglets and feeders were weighed for calculation of average daily gain (ADG), average daily feed intake (ADFI) and feed conversion ratio (FCR) on d 14 or d 28 of the experiment. Faecal scores were evaluated daily, and expressed as percentage for a period of two or four weeks. The severity of scours (1-5) based on Zhang and Guo (2007) were assigned daily by a person unaware of the dietary treatments (1 - hard faeces, rarely seen; 2 - no scours, normal consistency of faeces formed; 3 - mild scours, soft, partially formed faeces; 4 - moderate scours, loose, semiliquid faeces; 5-watery faeces). Values of each pen were subsequently averaged for a period of two or four weeks.

Table 1. Composition of basal diets

Ingredients	Amount, %		Calculated composition		
	1-14 d	15-28 d		1-14 d	15-28 d
Maize	55	55	DE, MJ/kg	14.23	14.15
Soyabean meal (44%)	14	14	CP, %	20.15	20.12
Extruded soyabean	15	15	Ca, %	0.85	0.82
Fish meal	3	3	Total P, %	0.634	0.7
Sprayed dried plasma proteins	3	-	Available P, %	0.463	0.534
Enzyme-hydrolysate fish powder	-	3	Methionine and		
Soyabean oil	1	1	cystine, %	0.731	0.716
Whey	5	5	Lysine, %	1.344	1.339
Limestone	0.65	0.5	Threonine, %	0.906	0.878
Dicalcium phosphate	1.1	1	Zn[2], mg/kg	137.4	139.1
Sodium chloride	0.35	0.35	Cu[2], mg/kg	16.2	15.5
Colistin (10%)	0.06	0.06			
Choline chloride (50%)	0.2	0.2			
L-lysine.HCl (78%)	0.28	0.28			
DL-methionine	0.034	0.034			
Threonine	0.04	0.04			
Vitamin and mineral premix[1]	1.0	1.0			
Wheat bran	0.286	0.536			

[1] mineral and vitamin premix supplied, per kg of final diet, IU: vit. A 5000, vit. D_3 450, vit. E 60; mg: Zn 100 ($ZnSO_4$), Cu 10 ($CuSO_4 \cdot 5H2O$), Fe 100 ($FeSO_4$), Mn 10 ($MnSO_4 \cdot H_2O$), Se 0.3 (Na_2SeO_3), I 0.5 (KI), vit. K 4.4, riboflavin 8.8, niacin 33, pantothenic acid 30, thiamin 2.5, pyridoxine 4.0; µg: vit. B_{12} 22, folic acid 900, biotin 200; [2] analysed

The use of piglets for this study was approved by the Animal Care Committee of China Agricultural University.

Sample collection

At the end of the experiment, one barrow that the body weight was near the average of the pen was selected randomly to collect samples. The selected piglets were sacrificed after anaesthetizing by intraperitoneal injection of pentobarbital sodium (40 mg/kg). The abdominal cavity was opened, the pancreas (approximately 10 g) was sliced from the same area stored in propylene bags and immediately frozen in liquid nitrogen for further analysis of digestive enzyme activities. On removal of the entire gastrointestinal tract, digesta samples were taken from the duodenum (10 cm from stomach), the jejunum (100 cm from stomach), the ileum (10 cm from ileo-caecal orifice) and the proximal colon of each piglet, immediately stored in propylene tubes, and frozen in liquid nitrogen immediately. All samples were stored at -80°C until analysis could be performed.

pH measurements

The pH of the intestinal contents from the duodenum, jejunum, ileum and colon was measured immediately after collection. All pH measurements were made on a pH meter, which was standardized with certified pH 4 and pH 7 buffer solutions. The sensitivity of the assay was 0.01.

Digestive enzyme activity analysis

The samples were thawed and homogenized (1:5 wt/vol) in distilled water at 0°C, centrifuged at 10 000 *g* for 10 min at 4°C, and the supernatant was used to determine the protein concentration, the activities of trypsin and chymotrypsin. The zymogens of trypsin and chymotrypsin in pancreas homogenates were activated by incubation in 0.1% enterokinase solution 1:1 (vol/vol) for 1 h at 37°C.

Trypsin activity was measured from the hydrolysis of p-nitroaniline from benzoyl-DL-arginine-p-nitroanilide (DL-BAPNA) (Gertler and Nitan, 1970). Units were expressed as nanomoles of p-nitroaniline released per min per g of protein. A similar method was used for the determination of chymotrypsin with N-glutaryl-L-phenylalanine-p-nitroanilide (GPNA) replacing BAPNA (Erlanger et al., 1966).

Analysis of the SCFA concentrations

The concentration of SCFA (acetic acid, propionic acid, isobutyric acid, butyric acid, isovaleric acid, valeric acid) was measured using gas chromatography (Zijlstra et al., 1977). Two hundred mg of chyme were suspended in sterile distilled water (1.6 ml) and metaphosphoric acid (0.2 ml) was added. 2-Mercapto isobutyric acid (0.2 ml) was added as an internal standard. The sample was mixed for 45 min with an orbital shaker and centrifuged for 15 min at 10 000 *g* at room temperature. One µl of the extract were injected onto the column of a Hewlett Packard 19091N-213 series chromatograph with a flame ionization detector, a cross-linked phenyl methyl silicone capillary column (30 m × 0.32 mm × 0.5 µm). The carrier gas was helium at 20 ml per min, the air flow was 450 ml per min and the hydrogen flow was 40 ml per min, the injector was heated to 200°C and the detector to 250°C.

Statistical analysis

Data were analysed by General Linear Model (GLM) procedure of SAS (2002). The main effects of TBZC, $CuSO_4$, and their interactions were analysed. At P values <0.05, differences were considered as significant. If a significant difference for main effects existed, differences among each treatment group were tested by Duncan's multiple-range test. Each pen served as the experimental unit. Scouring data was analysed after being arcsin transformed. Actual scouring data listed in the table, but SEM was for the transformed data.

RESULTS

Growth performance. The effect of TBZC and $CuSO_4$ addition on body weight gain and feed consumption was shown in Table 2. The supplementation of TBZC had a clear effect on ADG (P≤0.05) for week 1-2 or week 1-4. The supplementation of TBZC increased ADG of weanling piglets (268 vs 251 for week 1-2, 335 vs 319.5 for week 1-4, respectively). TBZC supplementation did not affect ADG during week 3-4. TBZC did not affect ADFI in any of the periods.

Table 2. Growth performance of weanling piglets fed high dietary concentrations of TBZC or $CuSO_4$[1]

Item	Zn, Cu, mg/kg	0		1500		SEM	P-value		
		0	250	0	250		Zn	Cu	Zn×Cu
1-2 week	ADG, g	229^b	272^a	260^a	276^a	5.7	0.050	0.003	0.139
	ADFI, g	377^b	407^{ab}	383^b	435^a	7.3	0.137	0.002	0.373
	FCR	1.65	1.50	1.48	1.59	0.038	0.549	0.777	0.061
3-4 week	ADG, g	352^b	429^a	391^a	402^a	8.2	0.237	0.001	0.046
	ADFI, g	612^c	706^a	624^{bc}	688^{ab}	14.0	0.953	0.004	0.582
	FCR	1.74	1.65	1.60	1.71	0.030	0.265	0.962	0.235
1-4 week	ADG, g	291^c	348^a	325^b	345^a	5.6	0.003	0.00	0.001
	ADFI, g	495^b	555^a	503^b	562^a	9.7	0.601	0.001	0.960
	FCR	1.70	1.60	1.55	1.63	0.025	0.231	0.823	0.057

[1] each value represents the mean of five pens of eight pigs (n=5); [a,b,c] means on the same row lacking a common superscript letters are different (P<0.05); TBZC - tetrabasic zinc chloride; $CuSO_4$ - copper sulphate; ADG - average daily gain; ADFI - average daily feed intake; FCR - feed conversion ratio

The addition of $CuSO_4$ had a clear effect on ADG and ADFI. ADG and ADFI were greater (P<0.01) in piglets fed diets containing 250 mg Cu/kg from $CuSO_4$ in any of the periods. During week 3-4 and week 1-4, an interaction between TBZC and $CuSO_4$ was found for ADG of weanling piglets (P=0.001). TBZC supplementation stimulated ADG of piglets fed on diets unsupplemented but not on supplemented with $CuSO_4$. During week 1-4, piglets fed diets containing 250 mg Cu/kg from $CuSO_4$ or both containing 250 mg Cu/kg from $CuSO_4$ and 1500 mg Zn/kg from TBZC had the greatest ADG, followed by the animals fed diets containing only TBZC, containing neither TBZC nor $CuSO_4$. None of the dietary treatments affected FCR in any of the periods.

Faecal scores and consistency. The effect of TBZC and $CuSO_4$ on faecal scores and consistency was shown in Table 3. There were no effect on faecal scores and consistency by supplementation of TBZC and/or $CuSO_4$ in the diets during nursery period.

Table 3. Faecal scores and consistency of weanling piglets fed high dietary concentrations of TBZC or $CuSO_4$[1]

Item	Zn, mg/kg	0		1500		SEM	P-value		
	Cu, mg/kg	0	250	0	250		Zn	Cu	Zn×Cu
Faecal consistency	week 1-2	2.12	2.12	2.04	2.12	0.0147	0.152	0.222	0.164
	3-4	2.08	2.10	2.05	2.06	0.0180	0.340	0.616	0.851
	1-4	2.10	2.11	2.04	2.09	0.0124	0.122	0.277	0.510
Faecal scores, %[2]	1-2	8.53	10.36	5.25	10.05	0.1986	0.345	0.114	0.446
	3-4	4.43	6.65	3.10	2.87	0.1708	0.154	0.557	0.466
	1-4	6.53	8.50	4.16	6.53	0.1524	0.156	0.161	0.893

[1] each value represents the mean of five pens of eight pigs (n=5); [2] faecal scores (%) were calculated as the percent of the total number of days when signs of scours were evident within the pen on a total number of days (14 d or 28 d); TBZC - tetrabasic zinc chloride; $CuSO_4$ - copper sulphate

pH. The effect of TBZC and $CuSO_4$ on intestinal pH was shown in Table 4. There was only a significant interaction ($P<0.05$) between TBZC and $CuSO_4$ for pH in the jejunum. The supplementation of TBZC and $CuSO_4$ reduced pH in the jejunum compared with the other dietary treatments.

Table 4. Gastrointestinal pH of weanling piglets fed high dietary concentrations of TBZC or $CuSO_4$[1]

Zn, mg/kg	0		1500		SEM	P-value		
Cu, mg/kg	0	250	0	250		Zn	Cu	Zn × Cu
Duodenum	5.53	5.92	5.88	5.78	0.303	0.693	0.517	0.064
Jejunum	6.18[a]	6.28[a]	6.37[a]	5.58[b]	0.413	0.187	0.080	0.029
Ileum	6.08	6.42	6.52	6.60	0.311	0.225	0.409	0.596
Colon	6.01	6.29	6.26	6.24	0.306	0.571	0.500	0.412

[1] each value represents the mean of five piglets, one pig per pen (n=5); [a,b] means on the same row lacking a common superscript letters are different ($P<0.05$); TBZC - tetrabasic zinc chloride; $CuSO_4$ - copper sulphate

Digestive enzyme activity. The effect of TBZC and $CuSO_4$ on pancreatic and intestinal digestive enzyme activities was shown in Table 5. There was a significant interaction between TBZC and $CuSO_4$ for trypsin activities in both pancreatic tissue homogenates (P<0.01) and duodenal contents (P<0.05) and for chymotrypsin activities in both pancreatic tissue homogenates (P<0.05) and jejunum contents (P<0.01) at 28 days. $CuSO_4$ supplementation reduced trypsin activities in duodenal contents and chymotrypsin activities in both pancreatic tissue homogenates and jejunum contents of piglets fed on the TBZC diet, but not in those without TBZC. The supplementation of TBZC increased (P<0.05) chymotrypsin activities in the duodenum contents (3.145 vs 2.095, respectively). Neither TBZC nor $CuSO_4$ addition had a significant effect on trypsin activities in the jejunum contents.

Table 5. Digestive enzyme activities (U/mg protein) in pancreas and in digesta of duodenum, and jejunum of weanling piglets fed high dietary concentrations of TBZC or $CuSO_4$[1]

Item	Zn, mg/kg	0		1500		SEM	P-value		
	Cu, mg/kg	0	250	0	250		Zn	Cu	Zn×Cu
Pancreas	trypsin	77.5[b]	96.6[a]	95.1[ab]	78.5[ab]	3.35	0.967	0.833	0.007
	chymotrypsin	3.10[b]	4.76[b]	8.04[a]	5.40[b]	0.587	0.005	0.564	0.022
Duodenum	trypsin	130.0[ab]	146.9[ab]	158.0[a]	113.6[b]	6.80	0.824	0.264	0.022
	chymotrypsin	2.08[b]	2.11[b]	3.80[a]	2.49[b]	0.262	0.030	0.156	0.143
Jejunum	trypsin	168.6	201.7	171.4	200.2	13.59	0.982	0.307	0.941
	chymotrypsin	1.64[c]	2.25[ab]	2.79[a]	1.86[bc]	0.141	0.072	0.417	0.002

[1] each value represents the mean of five piglets, one pig per pen (n=5); [a,b,c] means on the same row lacking a common superscript letters are different (P<0.05); TBZC - tetrabasic zinc chloride; $CuSO_4$ - copper sulphate

Short-chain fatty acids. The effect of TBZC and $CuSO_4$ on the SCFA concentrations in the ileum and colon was shown in Table 6. The supplementation of TBZC and $CuSO_4$ had no effect on ileum SCFA concentrations. There was an interaction between TBZC and $CuSO_4$ (P<0.05) in total SCFA concentrations in the colon. Piglets offered diets containing 250 mg/kg Cu from $CuSO_4$ had significantly increased (P<0.01) total SCFA concentrations in the colon. The piglets offered diets containing TBZC had a significantly higher total SCFA concentrations in the colon than the TBZC-free diets (P<0.01). TBZC inclusion increased (P<0.05) the proportion of acetic acid (15.103 vs 11.878, respectively) and propionic acid (6.188 vs 5.059, respectively), and significantly reduced (P<0.05) the proportion of isobutyric acid (1.470 vs 1.830, respectively) and isovaleric acid (0.171 vs 0.203, respectively) in the colon. Piglets offered diets supplemented with $CuSO_4$ had significantly increased (P<0.01) the proportion of acetic acid (14.89 vs 12.09, respectively), propionic acid (6.625

vs 4.621, respectively) and isobutyric acid (1.904 vs 1.396, respectively) in the colon. The supplementation of TBZC and $CuSO_4$ had no effect on colon valeric acid concentration and the ratio of acetic acid to propionic acid.

Table 6. SCFA concentration (mg/g fresh content) in ileum and colon of weanling piglets fed high dietary concentrations of TBZC or $CuSO_4$[1]

Item	Zn, mg/kg	0		1500		SEM	P-value		
	Cu, mg/kg	0	250	0	250		Zn	Cu	Zn×Cu
Ileum	total SCFA[2]	3.197	3.212	3.498	3.595	0.258	0.572	0.885	0.976
	acetic acid	1.965	2.090	2.193	2.247	0.138	0.494	0.749	0.899
	propionic acid	0.947	0.934	0.979	0.990	0.090	0.812	0.998	0.947
	isobutyric acid	ND	ND	ND	ND				
	butyric acid	0.236	0.180	0.266	0.281	0.034	0.356	0.771	0.614
	isovaleric acid	0.049	0.053	0.041	0.052	0.003	0.420	0.191	0.526
	valeric acid	ND	ND	ND	ND				
	acetic : propionic acid ratio	2.184	2.396	2.329	2.305	0.089	0.886	0.607	0.519
Colon	total SCFA	17.166^{c}	24.629^{ab}	23.265^{b}	26.493^{a}	0.481	0.001	<0.001	0.043
	acetic acid	9.789^{b}	13.966^{a}	14.390^{a}	15.815^{a}	0.455	0.003	0.007	0.150
	propionic acid	3.723^{b}	6.394^{a}	5.519^{a}	6.856^{a}	0.236	0.029	0.001	0.177
	isobutyric acid	1.571^{bc}	2.090^{a}	1.222^{c}	1.719^{ab}	0.068	0.018	0.002	0.936
	butyric acid	1.564	1.659	1.641	1.607	0.072	0.935	0.832	0.659
	isovaleric acid	0.210^{a}	0.196^{ab}	0.156^{b}	0.187^{ab}	0.007	0.035	0.553	0.117
	valeric acid	0.309	0.323	0.337	0.309	0.024	0.884	0.890	0.673
	acetic : propionic acid ratio	2.657	2.223	2.601	2.471	0.118	0.692	0.251	0.530

[1] each value represents the mean of five piglets, one pig per pen (n=5); [2] total SCFA - acetic acid + propionic acid + isobutyric acid + butyric acid + isovaleric acid; + valeric acid; [3] ND - not detected
[a,b,c] means on the same row lacking a common superscript letters are different (P<0.05);
TBZC - tetrabasic zinc chloride; $CuSO_4$ - copper sulphate; SCFA - short-chain fatty acid

DISCUSSION

Results from the current experiment indicated that supplementation of TBZC increased ADG. This result was consistent with previous reports that supplementation of Zn at 1500 mg/kg diet from TBZC enhanced the growth performance of weanling piglets (Mavromichalis et al., 2001; Zhang and Guo, 2007). $CuSO_4$ is commonly added into weanling piglets diets at concentrations above the nutritional requirement, because high level of $CuSO_4$ had been shown to have growth stimulatory properties for weanling piglets (Armstrong et al., 2004). Results similar to these in our experiment, which inclusion of $CuSO_4$ to weanling piglet diets increased ADG, were also observed. Limited study is available to document or explain the interactions between TBZC and $CuSO_4$ in weanling piglets. Smith et al. (1997) found an interaction between ZnO and $CuSO_4$ for ADG from d 14 to 28 after weaning. A regional study conducted by Hill et al. (2000) found an interaction between Zn (ZnO) and Cu ($CuSO_4$) for growth performance, and responses to Zn and Cu were independent and not additive in the current experiment. There was only an interaction between TBZC and $CuSO_4$ for 1-4 week ADG. This small difference may be attributed to physical characteristics of Zn sources (ZnO or TBZC). Zhang and Guo (2007) reported that TBZC was completely soluble in 0.4% HCl, 2% citric acid and neutral ammonium citrate, but the solubility value of ZnO was 94, 70 and 61% in 0.4% HCl, 2% citric acid and neutral ammonium citrate, respectively.

Dupont et al. (1994) reported that the inclusion of Zn or Cu to cell culture could inhibit the activity of haemolysin from *Serpulina hyodysenteriae*, an important virulence factor in the pathogenesis of pig dysentery. Previous research reported that high levels of dietary Zn reduced the incidence and severity of diarrhoea, and improved faecal consistency after weanling (Schell and Kornegay, 1996; Hill et al., 2000). Recently, Zhang and Guo (2007) reported that the faecal scores and consistency were reduced in weanling piglets fed high level of TBZC. Hill et al. (2000) found a significant Zn and Cu effects and a Zn x Cu interaction on faecal consistency. They reported that high Zn, high Cu, or the combination of high Zn and Cu resulted in firmer stools than in piglets fed only adequate Zn and Cu. The lack of a reduction in incidence and severity of diarrhoea for TBZC or $CuSO_4$ in this experiment may be explained by the diet composition, and by the excellent health status of the weanling piglets fed the control diet.

Maintenance of optimal pH in the intestine is necessary for optimal digestive enzyme functionality. In the current study, results showed that neither TBZC nor $CuSO_4$ had significant effects on the pH of duodenum, ileum and colon contents. However, there was a significant interaction between TBZC and $CuSO_4$ for pH in the jejunum contents. TBZC and $CuSO_4$ synergically reduced pH of the jejunum contents. Feeding high level of TBZC and $CuSO_4$ resulted in pH reduction in jejunum, promoted one of the conditions which often associated with an optimal intestine ecosystem (Jensen, 1998). Changes in HCl secretion by stomach that regulate the pH may contribute to no effect of TBZC and (or) $CuSO_4$ on pH of the duodenum contents. Ileal pH returned to normal ranges more quickly than jejunal pH when they were perfused with solutions of different pH (Hurwitz and Bar, 1968) which may explain why TBZC and (or) $CuSO_4$ did not affect the pH of the ileum contents. In addition, the total SCFA in ileum was not affected by TBZC and $CuSO_4$. The effect of TBZC and $CuSO_4$ on intestinal pH may warrant further investigation.

Supplementation of TBZC in weanling piglet diet resulted in marked increases in the activity of chymotrypsin in the pancreatic tissue homogenate and intestinal contents. This result is in agreement with Hedemann et al. (2006) that feeding 2500 mg/kg Zn from ZnO increased the chymotrypsin activity. After weaning, a reduction in the activity of pancreatic enyzmes had been reported (Owsley et al., 1986). The activity of pancreatic digestive enzymes in intestinal contents had been used to evaluate the pancreatic secretion (Hedemann and Jensen, 2004). In this experiment, TBZC stimulated the synthesis and secretion of pancreatic chymotrypsin. This may be associated with the growth-promoting effect of TBZC. The lack of effect of TBZC on the activity of trypsin is contrary to the results obtained in rats where feeding large doses of ZnO resulted in increasing the activity of trypsin in pancreatic tissue as well as intestinal contents (Szabó et al., 2004). However, Hedemann et al. (2006) reported that the activity of trypsin in pancreatic tissue homogenates but not small intestinal contents was elevated after feeding a high dietary concentration of ZnO. The discrepancy may be attributed to Zn source and animals used in the experiments. The lack of effect of $CuSO_4$ on the enzyme activities in this experiment is in agreement with previous reports (Luo and Dove, 1996; Hedemann et al., 2006). Surprisingly, the addition of $CuSO_4$ to the diet containing 1500 mg Zn/kg TBZC had a negative effect on trypsin activities in duodenal contents and chymotrypsin activities in both pancreatic tissue homogenates and jejunum contents. This result warrants further investigation.

SCFA formation by intestinal bacteria is regulated by environmental, dietary and microbiological factors. The majority of SCFA in the gut are derived from bacterial breakdown of complex carbohydrates as well as proteins and peptides (MacFarlane and MacFarlane, 2003). TBZC and (or) $CuSO_4$ inclusion increased total SCFA concentrations in colon. These augments are mainly due to an increase of acetic and propionic acid concentrations in colon. The lack of effect on the ratio of acetic to propionic acid by TBZC and (or) $CuSO_4$ suggests that the ratio of carbon to nitrogen in colon was not affected by TBZC and (or) $CuSO_4$. The trophic effects of SCFA on epithelial cell proliferation had been documented *in vivo* studies with rats (Frankel et al., 1994). Campbell et al. (1997) documented that the greater total SCFA production may result in a decrease in mucosal atrophy by normalizing cell proliferation in the mucosa of rats. Dietary incorporation of TBZC and (or) $CuSO_4$, by producing a greater concentration of total SCFA, may be beneficial in improving gastrointestinal health. Increased SCFA production may result in additional energy available for the weanling piglets. Isobutryic and isovaleric acid are formed from amino acid fermentation (MacFarlane and MacFarlane, 2003). In such fermentation, toxic metabolites such as ammonia and amines were formed. TBZC reduced the concentrations of isobutryic and isovaleric acid in colon, and ultimately may improve intestinal health.

CONCLUSIONS

In conclusion, supplemented weanling piglet diet with tetrabasic zinc chloride (TBZC) or CuSO4 increased average daily gain during the first 4 week after weaning. The combination of TBZC and $CuSO_4$ did not result in an additive response. It was shown that TBZC stimulated the synthesis and secretion of pancreatic chymotrypsin and may promote intestinal health. This finding may partly explain the beneficial effects of TBZC on growth performance of weanling piglets.

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