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EFFECT OF DIETARY ORGANIC ZINC SOURCES ON GROWTH PERFORMANCE, INCIDENCE OF DIARRHOEA, SERUM AND TISSUE ZINC CONCENTRATIONS, AND INTESTINAL MORPHOLOGY IN GROWING RABBITS

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Abstract: This study was conducted to evaluate the effect of dietary organic zinc (Zn) sources on growth performance, the incidence of diarrhoea, serum and tissue Zn concentration, and intestinal morphology in growing rabbits. A total of 120 New Zealand White rabbits aged 35 d and with an initial body weight of 755±15 g, were randomly divided into 4 treatment groups for a 49 d feeding trial. Dietary treatments were designed with different Zn supplements as follows: (1) Control group: 80 mg/kg Zn as $ZnSO_4$; (2) ZnLA group: 80 mg/kg Zn as Zn lactate; (3) ZnMet group: 80 mg/kg Zn as Zn methionine; (4) ZnGly group: 80 mg/kg Zn as Zn glycine. The results showed that, when compared with rabbits fed $ZnSO_4$, supplementation with ZnLA improved (P<0.05) growth performance and led to a lower (P<0.01) incidence of diarrhoea. ZnLA supplement increased the liver Zn concentration (58.97 *vs.* 46.59 mg/g; P<0.05) compared with rabbits fed $ZnSO_4$. Supplementing with ZnLA increased duodenum villi height (681.63 *vs.* 587.14 µm, P<0.05) and decreased duodenum crypt depth (141.69 *vs.* 168.91 µm; P<0.05) when compared to that of the control group. However, experimental results obtained ZnMet and ZnGly supplementation were no significantly different to $ZnSO_4$, except that feeding ZnMet led to higher (P<0.05) Zn concentrations in serum and liver than rabbits fed $ZnSO_4$. The results indicated that supplementation with 80 mg/kg Zn as ZnLA could improve growth performance, increase liver Zn concentration and enhance duodenum morphology, while reducing the incidence of diarrhoea in growing rabbits.

Key Words: zinc, growth performance, health, body Zn concentration, intestinal morphology, rabbit.

INTRODUCTION

Zinc is a component of numerous enzymes and is involved in the biosynthesis of nucleic acids and in cell division processes. It also has various beneficial effects on physiological functions, such as acid base balance, nutrient metabolism and immunity protection (Gaither and Eide, 2001; Hendy *et al.,* 2001). Frequently, Zn is used in the diets of pigs and broilers to enhance growth performance (Amen and Daraji, 2011; Wang *et al.,* 2012). A previous study reported that soybean meal and wheat bran were major ingredients of rabbit diets, which were rich in phytate and had an antagonistic effect on available Zn (Baker and Halpin, 1988). However, supplemental Zn could compensate for dietary Zn complexes with phytic acid in feed ingredients. The proposed Zn requirements for rabbits vary from 25 to 60 mg/kg (Mateos *et al.,* 2010). Better weight gain and feed intake were observed in rabbits receiving a diet supplemented with 90 mg/kg Zn (Hossain and Bertechini, 1993). Similarly, a previous work demonstrated that growing rabbits responded positively to 100 mg/kg of supplemental Zn, in terms of a significant improvement in live body weight gain (Nessrin *et al.,* 2012).

It is generally accepted that both organic and inorganic Zn can be used in animal diets to promote growth performance. In addition, pharmacological levels of Zn are frequently included in the first diet of pigs after weaning (Hahn and

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Baker, 1993). However, the use of high concentrations of Zn has raised some environmental concerns due to low Zn retention rates and the bioavailability of inorganic Zn (Case and Carlson, 2002). Up until now, organic Zn has been considered as an alternative to inorganic Zn in the diets of pigs and broilers, because of its better absorption and efficiency of use (Downs *et al.,* 2000; Wang *et al.,* 2010). Moreover, a previous study showed that inorganic forms of Zn differed in their bioavailability and efficacy in rabbits (Ferreira *et al.,* 2002). However, no recent trials have been conducted in rabbits to study the response to diets supplemented with organic Zn.

The objectives of this study were to investigate the effect of dietary organic Zn sources on growth performance, incidence of diarrhoea, serum and tissue Zn concentrations, and intestinal morphology in growing rabbits.

MATERIALS AND METHODS

Materials

In this study, experimental Zn sources, $ZnSO_4 \cdot H_2O$ (34.5% Zn as $ZnSO_4$), ZnLA (21.5% Zn as Zn lactate), ZnMet (17.5% Zn as Zn methionine) and ZnGly (22.0% Zn as Zn glycine) were provided by Sichuan Animtech Feed Co., LTD. Chengdu, China. ZnLA is a zinc lactic acid complex. ZnMet and ZnGly are both zinc amino acid complexes.

Animals and experimental design

All procedures were approved by the Institutional Animal Care and Use Committee of Sichuan Academy of Animal Science. Experimental work was done following guidelines for animal experimentation at the National Institute of Animal Health.

A total of 120 New Zealand White rabbits, aged 35 d and with an initial body weight of 755±15 g, were randomly divided into 4 treatment groups with 5 replicates and 6 rabbits per replicate. Rabbits were housed in the same building and placed in stainless steel cages (60×60×45 cm). There were 2 rabbits (1 male and 1 female) in each cage, and 3 cages for each replicate. The 4 dietary treatments were developed by supplementing the basal diet with the following concentrations and sources of Zn (1) Control group: 80 mg/kg Zn as $ZnSO_4$; (2) ZnLA group: 80 mg/kg Zn as ZnLA; (3) ZnMet group: 80 mg/kg Zn as ZnMet; (4) ZnGly group: 80 mg/kg Zn as ZnGly. The basal diet used in the experiment was formulated to meet or exceed the nutrient requirements recommended by the National Research

Table 1: Ingredients composition and nutrient levels of diet on air-dry basis.

Ingredients	%	Nutrient composition[b]	
Alfalfa meal	31.0	Digestible energy, MJ·kg^{-1}	10.50
Maize	28.0	Crude protein (%)	16.20
Wheat bran	21.5	Crude fibre (%)	12.80
Soybean meal	11.0	Ca (%)	1.00
Rapeseed meal	3.0	P (%)	0.60
Silkworm chrysalis	2.0	Lysine (%)	0.98
Calcium phosphate tribasic	1.0	Methionine + cystine (%)	0.63
Limestone	0.7		
Salt (NaCl)	0.5		
L-Lysine-HCl	0.2		
DL-Methionine	0.1		
Vitamin-mineral premix[a]	1.0		

[a]Provided per kg of diet: vitamin (vit.) A 10000 IU, vit. D_3 1000 IU, vit. E 20.00 mg, vit. K_3 1.00 mg, vit. B_1 2.00 mg, vit. B_2 2.00 mg, vit. B_6 1.00 mg, niacin 50.00 mg, calcium pantothenic 20.00 mg, folic acid 0.10 mg, biotin 0.20 mg, choline chloride 300 mg, vit. B_{12} 10.00 µg, Fe 50.00 mg, Cu 3.00 mg, Mn 8.50 mg, I 0.20 mg, Se 0.05 mg.
[b]Digestible energy based on calculated values, others were analysed values.

Council (NRC, 1977). Formulation and nutrient composition of the basal diet are presented in Table 1. The diets were mixed, pelleted and offered to the rabbits at 8:00 and 17:00 every day. Rabbits were raised for 49 d after a 7-d adaptation period. Throughout the experiment, rabbits were allowed *ad libitum* access to feed and water.

Measurements and chemical analyses

Growth performance

Individual body weight and feed intake per cage were recorded at the beginning and at the end of the growth trial. The feed conversion ratio was calculated using the average daily gain (ADG) and the average daily feed intake (ADFI) of rabbits. The ADG, ADFI and feed intake to gain (F/G) ratio data were corrected for dead rabbits in each cage.

Incidence of diarrhoea

During the growth experiment, rabbits were closely observed twice a day and all clinical signs of diarrhoea were carefully recorded. The clinical signs were scored from 0 to 3 (0=absence or normal; 1=feed intake disturbances or amorphous faeces; 2=light diarrhoea or abdominal swelling; 3=acute or advanced diarrhoea, constipation (caecal impaction), and presence of mucus in faeces) (Zhang *et al.,* 2013). When the score was 2 or 3, diarrhoea was recorded once. The incidence of diarrhoea was calculated as follows: Incidence of diarrhoea=summation of daily diarrheal events in each treatment/(number of rabbits in the treatment group×number of trial days)×100%.

Serum and tissue Zn concentrations

At the end of the growth experiment, ten randomly selected rabbits from each treatment fasted for 12 h and were then slaughtered under general anaesthesia with pentobarbital. Blood samples were drawn into 10-mL tubes by jugular venepuncture. Serum was obtained by centrifugation of the blood samples at 3000 rpm for 30 min at 5°C and then stored at –20°C until analysis for Zn concentration. Muscle samples from the left femur and liver samples were excised from rabbits and immediately stored at –20°C until analysis for Zn concentration. The concentrations of Zn in serum, liver and muscle of rabbits were measured using an atomic absorption spectrophotometer (AA-6300, Shimadzu, Tokyo, Japan) according to procedures outlined by Wedekind *et al.* (1992).

Intestinal morphology

The 3-cm long segments of duodenum, jejunum and ileum from ten rabbits in each group were collected and immediately fixed using neutralised 10% (v/v) formalin. Fixed tissue was washed with running water and dehydrated for paraffin embedding. Paraffin sections (5-µm thick) were deparaffinised and stained with haematoxylin-eosin. The measurements of villi height (VH) and crypt depth (CD) were performed using a light microscope (Nikon Eclipse E400 microscope, Nikon, Tokyo, Japan) and a system that analyses computerised images (Motic Image Plus 2.0, Motic China Group Co., Hong Kong). The height of 30 villi and the depth of 30 crypts from each intestinal segment were measured and the mean was obtained for each group and intestinal segment .

Statistical analysis

The obtained data were presented as means±standard errors (SD) and analysed using the one-way ANOVA test and Tukey test. The incidence of diarrhoea in every 2 experimental groups was analysed using a U-Test. All statistical analyses were performed using the SAS statistical package (SAS Institute, NC, USA). *P*-value <0.05 was considered statistically significant.

RESULTS

Effect of organic Zn sources on growth performance

The growth performance of rabbits is shown in Table 2. When compared with feeding $ZnSO_4$, supplementation with ZnLA improved ADG (P<0.05), ADFI (P<0.05) and F/G (P<0.05) of rabbits during the fattening period. However, there was no difference in growth performance between $ZnSO_4$, ZnMet and ZnGly treatments (P>0.05).

Table 2: Effect of dietary organic Zn on growth performance in growing rabbits.

Items	Control group	ZnLA group	ZnMet group	ZnGly group	*P*-value
Initial weight (g)	757±14	756±9	754±13	752±10	0.801
Final weight (g)	1979±76[a]	2166±64[b]	2110±77[ab]	2101±53[ab]	0.032
ADG (g)	24.9±1.3[a]	28.8±1.3[b]	27.7±1.9[ab]	27.5±1.9[ab]	0.024
ADFI (g)	100.4±3.2[a]	106.3±2.1[b]	105.6±2.9[ab]	105.2±2.4[ab]	0.028
F/G	4.03±0.17[b]	3.69±0.10[a]	3.82±0.14[ab]	3.82±0.18[ab]	0.036

Control group: 80 mg/kg Zn as $ZnSO_4$; ZnLA group: 80 mg/kg Zn as ZnLA; ZnMet group: 80 mg/kg Zn as ZnMet; ZnGly group: 80 mg/kg Zn as ZnGly. Data are presented as means±standard deviation. ADG: average daily gain; ADFI: average daily feed intake; F/G: feed intake to gain ratio. Initial weight: rabbits aged 35 d; Final weight: rabbits aged 91 d.
[ab]In the same row, values with different letter superscripts indicate significant difference (P<0.05).

Effect of organic Zn sources on the incidence of diarrhoea

The incidence of diarrhoea in rabbits due to dietary treatments is shown in Figure 1. When compared with feeding $ZnSO_4$, supplementation with ZnLA reduced the incidence of diarrhoea by 48% (P<0.05) throughout the experiment. However, there was no difference in the incidence of diarrhoea between $ZnSO_4$, ZnMet and ZnGly treatments (P>0.05).

Effect of organic Zn sources on serum and tissue Zn concentrations

Concentrations of Zn in rabbit serum, liver and muscle are shown in Table 3. As compared with rabbits fed $ZnSO_4$, supplementation with ZnMet increased serum Zn concentrations by 40% (P<0.05); and supplementation with ZnLA, ZnMet and ZnGly increased liver Zn concentrations by 27% (P<0.05), 30% (P<0.05) and 24% (P<0.05), respectively. However, no significant effect was observed on muscle Zn concentration between the dietary treatments (P>0.05).

Effect of organic Zn sources on intestinal morphology

Small intestine morphology of rabbits is shown in Table 4. As compared with rabbits fed $ZnSO_4$, supplementation with ZnLA increased duodenum VH by 16% (P<0.05), decreased duodenum CD by 16% (P<0.05), and increased VH/CD by 38% (P<0.05). Dietary Zn sources had no significant effect on VH, CD or VH/CD of the jejunum and the ileum (P>0.05).

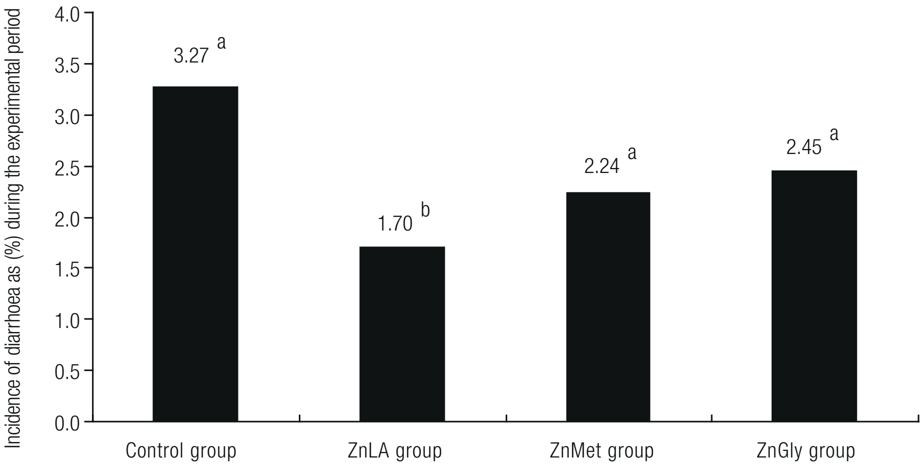

Figure 1: Effect of dietary organic Zn on incidence of diarrhoea in growing rabbits .Control group: 80 mg/kg Zn as $ZnSO_4$; ZnLA group: 80 mg/kg Zn as ZnLA; ZnMet group: 80 mg/kg Zn as ZnMet; ZnGly group: 80 mg/kg Zn as ZnGly. Different letter superscripts indicate significant difference (P<0.01).

Table 3: Effect of dietary organic Zn on serum and tissue Zn concentrations in growing rabbits.

Items	Control group	ZnLA group	ZnMet group	ZnGly group	*P*-value
Serum (mg/L)	3.48±0.67[a]	4.74±0.80[ab]	4.86±0.32[b]	4.30±0.59[ab]	0.041
Liver (mg/g)	46.59±3.19[a]	58.97±4.79[b]	60.77±4.73[b]	57.87±5.31[b]	0.030
Muscle (mg/g)	16.57±2.07	17.13±1.36	17.17±1.67	16.80±1.33	0.663

Control group: 80 mg/kg Zn as $ZnSO_4$; ZnLA group: 80 mg/kg Zn as ZnLA; ZnMet group: 80 mg/kg Zn as ZnMet; ZnGly group: 80 mg/kg Zn as ZnGly. Data are presented as means±standard deviation.
[ab]In the same row, values with different letter superscripts indicate significant difference ($P<0.05$).

DISCUSSION

Zinc is an essential trace element for rabbits. It was reported that feeding 3000 mg/kg Zn as ZnO to weaning pigs increased growth performance and reduced scouring immediately after weaning (Hahn and Baker, 1993). It was demonstrated that feeding weaning pigs a diet containing 250 mg/kg Zn as ZnMet increased growth performance in a similar way to pigs fed a diet containing 2000 mg/kg Zn as ZnO (Ward *et al.,* 1996). Moreover, it was shown that growth performance of pigs fed a diet containing 300 or 500 mg/kg Zn as Zn-Polysaccharide (Zn-PS) did not differ from that of pigs fed 2000 mg/kg Zn as ZnO; however, feeding 300 mg/kg Zn as Zn-PS decreased Zn excretion by 76% compared with feeding 2000 mg/kg Zn as ZnO (Buff *et al.,* 2005). In addition, the adverse effect of high Zn intake on copper availability has to be considered (Maret and Sandstead, 2006). Due to the environmental impact of Zn, the dietary requirement of Zn for rabbit growth was established at 25 to 60 mg/kg (Mateos *et al.,* 2010). However, further improvement in rabbit performance is possibly associated with extra inclusion of Zn beyond the recommended dose (Hossain and Bertechini, 1993; Nessrin *et al.,* 2012). In the present study, dietary supplementation with 80 mg/kg Zn as ZnLA significantly increased ADG, ADFI and the feed conversion ratio of rabbits compared to those fed a $ZnSO_4$ diet. In addition, growth performance did not vary significantly using dietary Zn from $ZnSO_4$, ZnMet and ZnGly. These results suggest that rabbits are more efficient at using dietary ZnLA than $ZnSO_4$, ZnMet or ZnGly.

Zinc is essential in immunocompetence in animals and the role of Zn as a component or activator of Zn-dependent enzymes explains most of the functional needs for Zn. Pharmacological levels of Zn from ZnO have been proven to enhance antibacterial function and reduce the incidence of diarrhoea (Hill *et al.,* 2001). It was suggested that ZnO was the only inorganic form of Zn that could produce these results (McCully *et al.,* 1995; Smith *et al.,* 1997). Lower dietary concentration of an organic Zn source was found to maintain growth performance at a level similar to that obtained with pharmacological concentrations of Zn as ZnO in weanling pigs (Ward *et al.,* 1996; Case and

Table 4: Effect of dietary organic Zn on intestinal morphology in growing rabbits.

Items	Control group	ZnLA group	ZnMet group	ZnGly group	*P*-value
Duodenum					
VH (μm)	587.14±29.43[a]	681.63±24.69[b]	640.19±19.78[ab]	634.32±24.07[ab]	0.039
CD (μm)	168.91±10.06[b]	141.69±8.85[a]	154.83±14.68[ab]	156.27±12.93[ab]	0.026
VH/CD	3.48±0.32[a]	4.81±0.53[b]	4.13±0.70[ab]	4.06±0.36[ab]	0.033
Jejunum					
VH (μm)	495.44±14.92	536.18±28.11	525.36±24.41	502.91±15.01	0.871
CD (μm)	126.74±14.47	113.13±13.00	118.72±10.66	121.71±17.74	0.692
VH/CD	3.91±0.45	4.74±0.56	4.43±0.26	4.13±0.55	0.570
Ileum					
VH (μm)	483.84±26.54	518.95±23.51	495.68±15.76	490.48±27.03	0.847
CD (μm)	130.91±15.06	119.14±10.73	120.27±13.60	125.78±12.17	0.683
VH/CD	3.70±0.63	4.36±0.97	4.12±0.50	3.90±0.59	0.409

Control group: 80 mg/kg Zn as $ZnSO_4$; ZnLA group: 80 mg/kg Zn as ZnLA; ZnMet group: 80 mg/kg Zn as ZnMet; ZnGly group: 80 mg/kg Zn as ZnGly. Data are presented as means±standard deviation. VH: villi height; CD: crypt depth.
[ab]In the same row, values with different letter superscripts indicate significant difference ($P<0.05$).

Carlson, 2002). However, there are no available data to confirm that organic Zn sources can be added to animals' diets at dietary concentrations lower than the pharmacological concentrations of ZnO commonly used in animal feeds and still reduce the incidence of diarrhoea. In the current study, as compared with rabbits fed $ZnSO_4$, supplementation with ZnLA reduced the incidence of diarrhoea in rabbits, but no significant differences were observed between $ZnSO_4$, ZnMet and ZnGly treatments. These results suggest that ZnLA can be used as a useful Zn source to reduce the incidence of diarrhoea in rabbits. This was possibly because dietary supplementation with ZnLA could benefit the development of intestinal mucosa in growing rabbits. Moreover, further studies are needed to investigate the exact mechanism by which ZnLA regulates the incidence of diarrhoea in rabbits.

Zinc is required for normal body metabolism and is present in animal plasma and tissue (Salim *et al.,* 2012). A previous study showed that the increase in plasma Zn concentration of pigs fed a high concentration of Zn from ZnMet was greater than that of pigs fed $ZnSO_4$ (Hahn and Baker, 1993). In the present study, the concentration of Zn serum significantly increased in rabbits receiving a ZnMet diet as compared with a $ZnSO_4$ diet. Plasma Zn concentration was monitored as a possible means to assess Zn bioavailability via a non-invasive procedure (Wedekind and Baker, 1990). Accordingly, our research pointed out that ZnMet as a Zn source for rabbits is more bioavailable than $ZnSO_4$. Moreover, our experimental results showed that, in comparison with the $ZnSO_4$ supplements, rabbits receiving diets supplemented with ZnLA, ZnMet and ZnGly showed higher liver Zn concentrations, even if no effect was noticed in Zn concentrations in muscle. These results suggest that organic Zn sources are more efficiently absorbed, and their retention in rabbits' livers is higher than that when using $ZnSO_4$. Furthermore, our experimental results are inconsistent with the work of Schell and Kornegay (1996), who reported that muscle tissue seemed to be a poor indicator of Zn status.

It is generally demonstrated that an unhealthy digestive tract often results in disturbances in digestive function and gastrointestinal disease (Pluske *et al.,* 1997; Tang *et al.,* 2009). Intestinal VH and CD are important indicators of the digestive health of the animal and are directly related to the absorptive capacity of the mucous membrane (Buddle and Bolton, 1992; Jia *et al.,* 2010). Morphological changes in the small intestine, such as shorter villi and deeper crypts, have been related to the presence of toxins, which are primarily related to a reduced feed intake (Gislason *et al.,* 1993; Pluske *et al.,* 1997). A previous research showed that feeding 3000 mg/kg Zn as ZnO to weanling pigs had an enteric effect of producing deeper crypts in the duodenum and a trend for longer villi (Carlson *et al.,* 1998). In the current study, increases in VH and decreases in CD were observed in the duodenum mucosa of the rabbits supplemented with ZnLA as compared with those given $ZnSO_4$. Rabbits supplemented with ZnLA had greater VH and lower CD in the duodenum mucosa, which may contribute to improved growth performance. However, in this study, there were no significant differences in the VH and CD of jejunum and ileum of rabbits receiving different zinc source diets.

CONCLUSION

In conclusion, this study showed that supplementation with 80 mg/kg Zn as ZnLA could improve growth performance and increase liver Zn concentration, as well as improve duodenum morphology and reduce the incidence of diarrhoea in growing rabbits. Our study indicated that ZnLA could be a good source of Zn dietary supplementation in young rabbits. Further studies are required to study the dose response between ZnLA and the effect on growth performance and the immunological capacity of growing rabbits.

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