PJN
ISSN 1680-5194

PAKISTAN JOURNAL OF NUTRITION

ANSInet
308 Lasani Town, Sargodha Road, Faisalabad - Pakistan
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Pakistan Journal of Nutrition 8 (4): 349-354, 2009
ISSN 1680-5194

Performance and Biochemical Responses in Early-Weaned Piglets Fed Diets with Different Protein and Zinc Levels

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Abstract: A 2×2 factorial experiment was used to investigate the interaction between dietary Crude Protein (CP) concentration (23 and17%) and zinc supplementation (100 and 3,000 mg/kg, from zinc oxide) on performance and biochemical responses in 32 crossbred piglets weaned at 21 days of age (4 groups of 8 each, 6.3±0.4 kg live weight). Pigs were offered four diets for 28 days after weaning, i.e., 23% CP +100 mg/kg Zn; 17% CP + 100 mg/kg Zn; 23% CP + 3,000 mg/kg Zn and 17% CP + 3,000 mg/kg Zn. There were no interactions between CP and Zn concentration on performances except for on ADG during 3 and 4 weeks. As CP content increasing, ADG, ADFI and Gain: Feed ratio (G:F) were increased across feeding period ($p<0.01$), however, the incidence of diarrhea was also increased ($p<0.05$). Supplemented those diets with pharmacological concentrations of Zn improved ADG ($p<0.01$), ADFI ($p<0.05$) and G:F ($p<0.01$) during first 2 weeks after weaning and decreased the incidence of diarrhea significantly ($p<0.01$), compared to the low-Zn diets. Dietary treatments also affected some enzymes and urea nitrogen in serum, but interactions were observed on AST and LDH from samples of 14 days only ($p<0.05$). In conclusion, pharmacological concentrations of Zn supplementation was favourable to ensure benefits of high-CP diets on performance of weanling pigs and this may due to its effect on lowing incidence of diarrhea at high CP concentrations. Pharmacological concentrations of Zn had no negative influence on pig health when period was 2 weeks post-weaning.

Key words: Early-weaned piglets, level of protein, zinc, growth performance, serum biochemistry, diarrhea

INTRODUCTION

Early weaning of piglets is usually association with depressed growth and greater incidence of diarrhea (Aherne *et al*., 1992) mainly due to an immature gastrointestinal tract and some change of gut morphology such as villous atrophy and digestive enzymes activities reduction (Lallès *et al*., 2004). Diets with high CP content are commonly used for early-weaned pigs. This kind of diet can improve growth performance of piglets (Yue and Qiao, 2008), but is always associated with high incidence of scouring (Ball and Aherne, 1987). Reducing concentration of dietary CP with supplementing essential AA decreased the incidence of diarrhea, but also caused poorer performance (Luo, 2005; Yue and Qiao, 2008).

Over the last decades, dietary antibiotics have often been used to promote growth and control diarrhea in piglets at this stage. Due to the progressive ban of dietary antibiotics, control of post-weaning diarrhea may require using strategies other than dietary antibiotics (Wierup, 2000). Pharmacological concentrations of zinc (from ZnO) are usually added to nursery pig diets during the early weaning period, which have been implicated in the prevention of diarrhea (Holm and Poulsen, 1996) and can improve performance of piglets (Kavanagh, 1992; Poulsen, 1992; Hahn and Baker, 1993). Serum biochemical parameters can reflect health status, condition of immunity and metabolism of animals, which are used widely in clinical situations. Since, protein and zinc were both important in improving growth of piglets, there was limited information about effects of diets with different levels of protein supplemented with or without pharmacological zinc on performance and blood parameters of early-weaned piglets.

The objectives of the present experiments were to determine the effects of reducing the dietary CP content from 23-17%, while supplementing with or without pharmacological concentrations of ZnO on growth performance, serum biochemical parameters and incidence of scouring in early-weaned pigs. The approach was to feed diets based on corn and soybean meal that were formulated to differ by 6 percentage units in CP but with an fixed ratio between vegetable and animal materials.

MATERIALS AND METHODS

Animals, housing and experimental design: The experiment was conducted with 32 crossbred piglets ([Yorkshire×Landrace]×Duroc), with an initial BW of 6.3±0.4 kg and weaning at 21 days of age. At weaning, piglets were allocated to 1 of 4 dietary treatments according to a 2×2 factorial arrangement, with 4 pens (2 piglets per pen) for each. Littermates of similar BW were randomly allocated to the treatments. Treatments contained equal numbers of females and castrated males.

All piglets were kept in stainless steel metabolism crates with slatted flooring. Pigs had free access to feed and water over the 28 days study. Room temperature, which was regulated by warmers, was initially set at 28°C and progressively decreased by 1°C each week. This study was approved by the Committee of Sichuan Agricultural University for Animal Care and Use.

Experimental diets: Four experimental diets based on corn-soybean meal were formulated with 2 levels of CP (23 or 17%), supplemented with two contents of Zn (100 mg Zn/kg diet or 3,000 mg/kg) from ZnO (Table 1). All the diets were supplemented with synthetic lysine (HCl-Lys) and only 2 diets containing low CP were supplemented with methionine. These diets were formulated to meet or exceed NRC (1998) recommendations for all other nutrients and were supplied in mash form.

Performance, blood sampling and assessment of severity of diarrhea: Piglets in each pen were weighed as a group on d 0 (weaning), 7, 14 and 28. Feed intake was determined weekly. Average Daily Gain (ADG), Average Daily Feed Intake (ADFI) and Gain: Feed ratio (G:F) were calculated per pen.

On 14 and 28 days, blood samples were collected from all experimental pigs via anterior vena cava puncture into 10 mL heparin-free glass tubes, which were centrifuged at 3,000 rpm (Centrifuge Model 0406-1, Shanghai Medical Instruments Corp. Ltd., Shanghai, China) for 10 min. Supernatant was gathered into Eppendorf tubes respectively and then immediately stored at -20 for later analysis.

Severity of diarrhea was monitored twice per day (i.e., at morning and dark). A faecal consistency score were used to evaluate the severity, which was based on a scale from 0-3 (0 = normal faeces, 1 = soft faeces, 2 = mild diarrhea and 3 = watery diarrhea). A co-worker who was not aware of pig treatments was asked to record the score. When score was 2 or 3, diarrhea was recorded once. Incidence of diarrhea was calculated according to following equation:

Table 1: Ingredients and chemical composition of experimental diets

	CP 23%		CP 17%	
	Zinc content		Zinc content	
Ingredients	100 mg/kg	3,000 mg/kg	100 mg/kg	3,000 mg/kg
Chemical composition				
Maize	50.36	50.36	63.31	63.31
Soybean meal	16.97	16.97	10.65	10.65
Wheat bran	3.23	3.23	2.03	2.03
Fish meal	4.24	4.24	2.67	2.67
Whey powder	6.00	6.00	6.00	6.00
Expanded soybean	7.68	7.68	4.83	4.83
Rapeseed meal	2.02	2.02	1.27	1.27
Corn gluten meal	2.83	2.83	1.78	1.78
Spray-dried blood cell meal	3.43	3.43	2.16	2.16
Soybean oil	0.79	0.79	1.82	1.82
Calcium carbonate	0.88	0.88	0.92	0.92
Dicalcium phosphate	0.6	0.6	0.98	0.98
Vitamin premix[1]	0.05	0.05	0.05	0.05
Mineral premix[2]	0.5	0.5	0.5	0.5
Choline chloride	0.15	0.15	0.15	0.15
Common salt	0.25	0.25	0.25	0.25
L-lysine •HCl[3]	0.02	0.02	0.56	0.56
DL-methionine[4]	—	—	0.07	0.07
Calculated content				
Crude protein[5] (%)	22.98	22.98	17.01	17.01
Digestive energy (MJ/kg)	14.24	14.24	14.24	14.24
Calcium (%)	0.80	0.80	0.80	0.80
Available phosphonium (%)	0.40	0.40	0.40	0.40
Lysine (%)	1.35	1.35	1.35	1.35
Methionine (%)	0.38	0.38	0.35	0.35

[1]Provided per kilogram of complete diet: 25,000 IU of vitamin A, 5,000 IU of vitamin D_3, 12.5 IU of vitamin E, 0.015 mg of vitamin B_{12}, 17.5 mg of nicotimic acid, 12.5 mg of D-pantothenic acid. [2]Provided per kilogram of complete diet: 100 mg of Fe (as $FeSO_4.7H_2O$), 6 mg of Cu (as $CuSO_4.5H_2O$), 4 mg of Mn (as $MnSO_4.H_2O$), 0.3 mg of Se (as Na_2SeO_3), 0.14 mg of I (as KI). [3]L-lysine •HCl containing 78.3% L-Lys. [4]DL-methionine containing 98% Met. [5]CP content was based on analysis

$$\text{Incidence of diarrhea} = \frac{\text{Numbers of pigs scouring}}{\text{Numbers of pigs in treatment}} \times 100\%$$

Analysis of biochemical parameters: Serum samples were analyzed for enzymatic activities of glutamic-oxal(o)acetic transaminase (AST), Glutamate-pyruvate Transaminase (ALT), Lactate Dehydrogenase (LDH) and Alkaline Phosphatase (AKP). The activity was determined with an automatic biochemistry analyzer (CL-7200, Shimadzu Instruments Manufacturing. Co., Ltd., Suzhou, China) using standard assay kits (Nanjing Jiancheng Biotechnology Co., Ltd, Jiangsu, China) and results were expressed as international units per liter as 25°C. Serum urea nitrogen concentration was measured using the same instrument.

Statistical procedures: Pen was the experimental unit for ADFI, ADG, G:F and biochemical parameters. Data was analyzed as a 2×2 factorial arrangement of treatments, with dietary CP and Zn content as the factors by General Liner Model using Univariate procedure. Statistical analysis of incidence of diarrhea was performed using the data from 8 dissections per treatment, with piglet as the experimental unit. Statistical significance was accepted at $p<0.05$ and tendencies for P between 0.1 and 0.05 were also presented. Statistical analysis was performed with SPSS 13.0.

RESULTS

Animal performance and incidence of diarrhea: There was no decease throughout the experiment. In Table 2, ADG, ADFI, G:F and incidence of diarrhea are presented. During first 2 weeks after weaning, either high-CP or low-CP diet supplemented 3,000 mg/kg Zn improved ADG ($p<0.01$), ADFI ($p<0.05$) and G:F ($p<0.01$), compared to those with 100 mg/kg Zn. Although for 3 and 4 weeks, low-CP supplemented with this content of Zn decreased ADG, ADFI and G:F (399.55-370.09, 832.08-795.73 and 0.48-0.47, respectively), 3,000 mg/kg Zn improved ADG ($p<0.01$) and G:F ($p<0.05$) across the period with no effect on ADFI ($p = 0.548$). The reduction in CP content decreased ADG and G:F in any period of the experiment ($p<0.01$), but only affected ADFI ($p<0.01$) during 4 weeks after weaning. As CP content increasing, the incidence of diarrhea was increased sharply ($p<0.05$), indicated that high-CP diet resulted in much more serious scouring of early-weaned piglets. But this situation was alleviated when pharmacological concentrations of Zn were supplemented to this diet. No interactions between Zn and CP content were found for those data except for their effect on ADG of 3 and 4 weeks ($p<0.05$).

Biochemical parameters: The effects of dietary treatment on biochemical parameters were shown in Table 3. During first 2 weeks after weaning, high-CP diet supplemented with 3,000 mg/kg Zn tended to increase concentration of ALT ($p=0.051$) but increased serum LDH ($p<0.01$), AKP ($p<0.01$) and decreased serum BUN ($p<0.01$), effects of which were similar with low-CP diet compared with that of 100 mg/kg Zn. High content of CP decreased serum LDH ($p<0.01$) and tended to lower ALT ($p = 0.097$) of piglets, but the tendency for effect on AST was different between the two content of Zn. Interactions between dietary CP and Zn concentration were found for AST and LDH ($p<0.05$) in this period and there was a tendency for AKP ($p = 0.08$). For last 2 weeks of this study, the effects of Zn on ALT ($p<0.01$), LDH ($p<0.05$) and AKP ($p<0.01$) were similar between high-and low-CP diets. Increasing CP content resulted in higher BUN ($p<0.01$) and tended to lower AST ($p = 0.076$). There was no interaction between CP and Zn levels on those parameters from 15-28 days after weaning, except a tendency for AKP ($p = 0.086$).

Table 2: Effect of dietary CP and Zn content on performance and incidence of diarrhea of piglets

	Level of zinc					p-value		
	100 mg/kg		3000 mg/kg					
Items	23% CP	17% CP	23% CP	17% CP	S.E.M	Zn	CP	Zn×CP
Initial BW (kg)	6.26	6.25	6.26	6.27	0.008	ns		
Final BW (kg)	15.56	14.00	16.24	14.20	0.085	0.023	<0.001	0.180
ADG (g/d)								
1 and 2 weeks	185.27	147.32	212.05	195.99	3.300	<0.001	0.001	0.123
3 and 4 weeks	470.98	399.55	500.89	370.09	6.294	0.986	<0.001	0.036
1-4 weeks	328.13	273.44	356.47	283.04	3.001	0.008	<0.001	0.144
ADFI (g/d)								
1 and 2 weeks	301.67	278.42	308.21	312.92	3.437	0.011	0.202	0.065
3 and 4 weeks	862.81	832.08	879.18	795.73	7.533	0.519	0.003	0.106
1-4 weeks	582.24	555.25	593.69	554.32	4.260	0.548	0.002	0.482
Gain:feed ratio								
1 and 2 weeks	0.62	0.53	0.69	0.63	0.011	0.002	0.005	0.504
3 and 4 weeks	0.55	0.48	0.57	0.47	0.006	0.676	<0.001	0.106
1-4 weeks	0.56	0.49	0.60	0.51	0.005	0.021	<0.001	0.402
Incidence of diarrhea (%)	25.00	15.63	9.38	6.25	1.426	0.001	0.049	0.295

Table 3: Effect of dietary CP and Zn content on biochemical parameters of piglets

	Level of zinc							
	100 mg/kg		3000 mg/kg			p-value		
Itams	23% CP	17% CP	23% CP	17% CP	S.E.M	Zn	CP	Zn×CP
Day 14								
ALT (IU/L)	21.00	22.25	31.50	35.50	2.743	0.051	0.641	0.806
BUN (m mol/L)	4.28	4.11	3.12	2.32	0.164	0.001	0.167	0.366
AST (IU/L)	35.00	38.25	49.75	33.50	1.602	0.145	0.065	0.010
LDH (IU/L)	583.00	610.75	645.75	841.00	17.016	0.001	0.007	0.030
AKP (IU/L)	235.50	229.50	317.75	383.75	9.424	<0.001	0.137	0.080
Day 28								
ALT (IU/L)	27.75	31.25	45.00	50.75	2.515	0.003	0.376	0.827
BUN (m mol/L)	4.38	2.70	5.00	3.13	0.159	0.126	<0.001	0.756
AST (IU/L)	31.00	42.00	39.50	47.50	2.445	0.178	0.076	0.764
LDH (IU/L)	1211.75	1135.25	1373.25	1525.50	57.296	0.033	0.747	0.338
AKP (IU/L)	187.75	152.50	291.00	305.00	6.585	<0.001	0.436	0.086

DISCUSSION

This experiment was conducted to evaluate the influence of dietary CP and Zn content on growth performance, incidence of diarrhea and biochemical parameters of piglets. We hypothesized that increase in dietary CP would improve the performance and result in more serious diarrhea, but supplementing pharmacological concentrations of Zn (from ZnO) may prevent or decrease incidence of diarrhea so as to gain a better performance of weaned piglets.

Diets with high content of CP improved overall growth performance, this is in close agreement with previous studies (Luo, 2005; Htoo *et al.*, 2007). The role of protein is very obvious, young animal need much more protein from diet for development because of the contrary between the immature gastrointestinal tract and fast growth, but high CP may increase microbial fermentation of undigested protein and encourage proliferation of pathogenic bacteria in the gastrointestinal tract (Ball and Aherne, 1987), which would cause scouring. It was anticipated that reducing dietary CP while balancing the diets for essential AA would support similar piglet performance (Hansen *et al.*, 1993; Le Bellego and Noblet, 2002). However, some reports were contrary to them. Nyachoti *et al.* (2006) reported that final BW and the overall ADG and ADFI were reduced by feeding diets containing lower CP compared with which containing 23% CP. Recently, Yue and Qiao (2008) showed that reducing CP level did not affect ADFI but resulted in poorer ADG and F:G of early-weaned pigs during first two weeks post-weaning. In this study, only two essential AA was considered, both of which met or exceeded recommendations of NRC (1998), but the ratio between vegetable and animal materials was the same within diets with different levels of CP. It was aimed to avoid the effects of crystalline AA supplemented, which may affect the responses estimated in this study and also to find out another strategy to improve performance of early-weaned piglets. Addition of 3,000 mg/kg Zn (from ZnO) also improved performance of young pigs fed with either high or low content of CP. It has been demonstrated that pharmacological concentrations of Zn (from ZnO) could improve growth performance of early-weaned piglets and decrease the incidence and severity of diarrhea (Holm, 1990; Hahn and Baker, 1993) and this positive effect happened when dose was 2,000-4,000 mg Zn/kg diet (Smith *et al.*, 1997; Hill *et al.*, 2000). In general, young pigs fed diets with high level of Zn had better performance than that of low level in this study, except for ADFI of 1-2 weeks. It was in our expectation that pigs received high-CP diet supplemented with 3,000 mg/kg Zn had the best performance (except for ADFI of 1 and 2 weeks), which may due to the prevention of Zn against diarrhea. Some mechanisms may explain why pharmacological concentrations of Zn had this effect, one of which was the inhibition of Zn on proliferation of pathogenic bacteria such as *Escherichia coli* (Broom *et al.*, 2003). This may explain when high-CP diet was supplemented with pharmacological concentrations of Zn, the incidence and severity of diarrhea was modified.

The amount of any single enzyme in serum indirectly reflects its concentration in the cell, extent of cell injury, normal cell death or apoptosis and its degradation in plasma (Kaneko *et al.*, 1997). ALT and AST are two of the most reliable markers of hepatocellular injury or necrosis. AST is known to present in various tissues like liver, heart, kidney, brain, pancreas and lungs. Therefore, the present of AST is a sensitive marker of soft tissue damage although, it is not an organ-specific enzyme (Boyd, 1983). Dietary CP may affect functions of liver and proper content of CP can prevent some diseases (Tanabe *et al.*, 2002). In our study, there was an interaction between CP and Zn content on AST of 1 and 2 weeks. During this period, High content of CP tended to decrease AST while did not affect ALT, indicated that higher content of CP may benefit for pig health, which was similar to other's report (Luo, 2005). But pharmacological concentrations of Zn increased AST in pigs fed with high-CP diet, compared with that of low-CP diet. Furthermore, Pharmacological concentrations of Zn increased content of ALT during 3 and 4 weeks after weaning, indicated that the supplement of Zn lasting long may have no benefit. However, the content of ALT of this study was in the normal range (10.5-45 IU/L) of animals (Lu, 1974).

AKP and LDH play a role in AA and carbohydrate metabolism and are sensitive to Zn. Their activities increase can enhance synthesis of protein. Zn increased activities of those 2 enzymes in our study, which was similar with others (Cai, 2004; Leng *et al.*, 2004), indicated that Zn may improve synthesis of protein which may explain the promotion of Zn on performance.

Urea excreted in urine is the main nitrogenous end-product from AA catabolism in pigs and plasma or serum urea concentrations may be indicative of excreted N in urine (Brown and Cline, 1974; Zervas and Zijlstra, 2002). In addition, Serum or plasma urea nitrogen can be used in various animal species to quantify N utilization and excretion rates (Kohn *et al.*, 2005). Lower blood urea nitrogen indicated higher availability of dietary nitrogen (Figueroa *et al.*, 2002; Owusu-Asiedu *et al.*, 2003). In the present study, the effect of CP level was different in two periods. In first 2 weeks post-weaning, CP level had no effect on serum urea nitrogen. This was contrast to Yue and Qiao (2008), who found that as CP level increasing, serum urea nitrogen was increased in piglets during first 2 weeks post-weaning. The discrepancies may be explained by differences in the age and the genetype of piglets used. In our study, (Yorkshire×Landrace)×Duroc piglets were weaned at 21 day of age, whereas Yue and Qiao (2008) used White×Landrace barrows weaned at 18 days. But during 3 and 4 weeks, serum urea nitrogen was lower in pigs fed with low-CP diet, compared to which consumed a higher CP diet. High Zn content decreased serum urea nitrogen during first 2 weeks after weaning, which was in agreement with others (Brown and Cline, 1974; Fang, 1999). Lower concentrations of serum urea nitrogen indicated a lower rate of protein catabolism and a higher deposition of protein, which may cause a better response of performance.

Conclusion: In our study, there were positive responses of performance and biochemical parameters in piglets when fed higher content of CP or Zn. High-CP diet did have a positive effect on performance and pharmacological Zn (from ZnO) could enhance this advantage by decreasing incidence of diarrhea without an injury to piglet health, such as soft tissue damage.

ACKNOWLEDGEMENT

This RESAERCH was supported by the Program for Changjiang Scholars and Innovative Research Team in University (IRTO555).

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