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Effects of Dietary Zinc Level and an Inflammatory Challenge on Performance and Immune Response of Weanling Pigs*

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ABSTRACT : Two experiments were conducted to determine the effect of dietary zinc level on growth performance and immune function in normal (Experiment 1) and immunologically challenged (Experiment 2) weanling pigs. Treatments consisted of the following: i) a corn-soybean meal basal diet containing 36.75 mg/kg total Zn, ii) basal diet+60 mg/kg added Zn as $ZnSO_4$, iii) basal diet+120 mg/kg added Zn as $ZnSO_4$. Each diet was fed to six pens of four pigs per pen (Exp. 1) or six pens of three pigs per pen (Exp. 2). In Exp. 1, the dietary zinc level had no effect on average daily growth (ADG), average daily feed intake (ADFI), or feed conversion ratio (FCR). Concentrations of tissue and serum zinc were not affected. Peripheral blood lymphocyte proliferation (PBLP) was not affected by dietary treatments. Supplementation of 120 mg/kg Zn decreased ($p<0.05$) the antibody response to bovine serum albumin (BSA) on d 7 compared with pigs fed the basal diet, but not on d 14. In Exp. 2, LPS challenge had no effect on ADG, ADFI and FCR in the entire trial (from d 0 to 21). LPS challenge significantly decreased ADG and ADFI ($p<0.01$) from d 7 to 14, but FCR was not affected. LPS challenge increased PBLP ($p<0.05$) and serum concentration of interleukin-1 (IL-1) ($p<0.01$), whereas the antibody response to BSA and serum concentration of interleukin-2 (IL-2) were not affected. Supplementation of Zn did not affect ADFI and FCR from d 7 to 14, but there was a trend for ADG to be enhanced with Zn supplementation ($p<0.10$). Supplementation of Zn tended to increase PBLP ($p<0.10$). Dietary treatment had no effect on the antibody response to BSA or concentrations of serum IL-1 and IL-2. Results indicate that the level of Zn recommended by NRC (1998) for weanling pigs was sufficient for optimal growth performance and immune responses. Zn requirements may be higher for pigs experiencing an acute phase response than for healthy pigs. (**Key Words :** Performance, Immune Response, Pig, Zinc, Immune Challenge)

INTRODUCTION

Zinc is a cofactor of >300 enzymes and is known to be essential for growth and development of all organisms. Zinc is involved in many metabolic processes and Zn deficiency has been demonstrated to reduce weight gain and feed intake, and cause parakeratosis or diarrhoea in young pigs (Miller et al., 1968; Whitenack et al., 1978). Zinc is also necessary for the normal function of the immune system. Zinc supplementation must be adjusted to the actual requirements of pigs because severe zinc deficiency leads to dysfunction of the immune system. Studies reveal that dietary requirements of trace minerals to optimize immune function may be higher than the requirements for growth (Klasing, 2001), yet studies in swine to determine effects of Zn on immunity are lacking (Johnson et al., 2001).

It is well documented that an immunological stress can cause growth suppression and decreased rate of lean tissue deposition (Johnson, 1997; Jacobi et al., 2006), which may increase economic loss for animal producers. Klasing et al. (1987) found decreased weight gain, feed intake, and efficiency of feed utilization in chicks that were repeatedly challenged with noninfectious agents. Infection decreased serum Zn and concomitantly increased hepatic and splenic Zn in Chicks (Linda et al., 1988). Metabolic shifts following immune challenge are brought about by interleukin-1 (IL-1) and tumor necrosis factor (TNF) produced by stimulated macrophages (Klasing, 1988). It has been shown that metabolic changes associated with infectious diseases or inflammatory processes can result in decreases in weight gain and feed efficiency. These changes

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Table 1. Composition and nutrient levels of the basal diet (as-fed basis)

Ingredient	%
Corn	58.74
Soybean meal	16.00
Extruded full-fat soybean	10.58
Whey	3.50
Fish meal	5.00
Soybean oil	2.70
$CaCO_3$	0.87
$CaHPO_4$	0.80
L-lys·HCl	0.30
DL-met	0.02
Threonion	0.11
Chloride cholin	0.1
Vitamins[a]	0.03
Salt	0.25
Minerals[b]	1.00
Nutrient levels	%
Digestible energy (MJ/kg)	14.11
Crude protein	20.65
Calcium	0.81
Phosphorus, total	0.60
Phosphorus, available	0.43
Lysine	1.19
Methionine	0.34
Threonine	0.74
Tryptophan	0.24
Zinc (mg/kg)	36.75

[a] Vitamin mixture supplied as the following (per kg basal diet): vitamin A , 15,000 IU; vitamin D_3, 3,000 IU; vitamin E, 7.5 IU; vitamin K_3, 1.5 mg; vitamin B_1, 0.6 mg; vitamin B_2, 4.8 mg; vitamin B_6, 1.8 mg; vitaminB_{12}, 0.009 mg; nicotinic acid, 10.5 mg; pantothenic acid, 7.5 mg; folic acid, 0.15 mg; biotin, 80.0 mg.

[b] Minerals mixture provide as the following (per kg basal diet): Fe as $FeSO_4·7H_2O$, 100 mg; Mn as $MnSO_4·H_2O$, 4 mg; Cu as $CuSO_4·5H_2O$, 6 mg; I as KI, 0.14 mg; Se as $NaSeO_3$, 0.30 mg.

[c] Zinc level is analytical value.

in metabolism suggest altered nutritional requirements during immunologic challenge. Nutrients are redistributed away from the growth process and toward support of immune system function (Beisel, 1977). Although knowledge about the field of zinc immunology has increased during the past years, it is still not clear whether Zn requirements following a period of immune stress are altered. The objective of this study was to investigate the effect of dietary Zn level on growth performance and immune response in normal and immunologically challenged pigs and to determine whether an inflammatory challenge (lipopolysaccharide injection) interacted with the Zn to affect performance and immune response.

MATERIALS AND METHODS

Experimental animals and design

The animal protocols for this research was approved by the Sichuan Agricultural University Animal Care and Use Committee. In Exp. 1, seventy-two crossbred pigs (Duroc×Landrance×Yorkshire) weaned at 28 d of age (6.76±0.47kg) were randomly allotted to one of three dietary treatments by initial BW. Treatments consisted of the following: i) a corn-soybean meal basal diet contained 36.75 mg/kg total Zn, ii) basal diet+60 mg/kg added Zn as $ZnSO_4$, iii) basal diet+120 mg/kg added Zn as $ZnSO_4$. Pigs were housed in 1.28×1.28 m pens with six replicates per treatment with four pigs per pen. Each pen was equipped with one feeder and one nipple waterer to allow pigs *ad libitum* access to feed and water. Room temperature was maintained at 25-28°C and a cycle of 12 h light:12 h dark were controlled. The basal diet (Table 1) was formulated to meet NRC (1998) requirements for all nutrients except zinc. Body weight and feed intake were measured weekly throughout the 21-d trial.

One randomly selected pigs per pen received a hypodermic injection of bovine serum albumin (BSA) (Sigma Chemical Inc., St. Louis, USA) 1 mg/kg of body weight on d 7 of the trial. BSA was dissolved in a 0.9% (wt/vol) NaCl solution. On d 7 and 14 after BSA injections, blood samples were taken by venipuncture and centrifuged (3,500×g for 10 min) to collect serum, and then stored at -20°C until analysis. *In vitro* lymphocyte proliferation was measured on d 21 of the trial in one selected pig per pen. 18 pigs (six pigs per treatment, one pig per pen) were killed by i.v. injection of 4 ml of sodium pentobarbital for evaluation of tissue and serum Zn concentrations. Liver, kidney, spleen, and phalanges were excised, and then frozen at -20°C until analysis.

In Exp. 2, the remaining 54 pigs continued to receive their dietary treatments. According to a 2×3 factorial arrangement that included *E. coli* lipopolysaccharide (LPS) challenge (with or without) and a dietary addition of Zn (0, 60, and 120 mg/kg Zn). Half replicates of each treatment were injected intraperitoneally with LPS (*Escherichia coli* serotype 055:B5, Sigma Chemical Inc., St Louis, MO, USA) at 200 μg/kg BW, and half were injected an equivalent amount of 0.9% (wt/vol) NaCl solution on d 7 of the trial. The LPS was dissolved in sterile 0.9% NaCl solution (500 mg LPS/L saline). At 3h after injection, blood samples (one pig per pen) were collected into vacuum tubes and centrifuged (3,500×g for 10 min) to collect serum, and then stored at -80°C until they were analyzed for interleukin-1 and interleukin-2 concentrations. Two days after the LPS or saline injection, lymphocytes were isolated from peripheral blood from one pig per pen. Furthermore, one pig per pen (pigs different from the pigs used for the analysis of serum samples and lymphocyte proliferation above) was injected intramuscularly with 1 mg/kg BW BSA to determine humoral immune response. The Blood samples

were collected on d 7 and 12 after the injection of bovine serum albumin. Serum was separated by centrifugation (3,500×g for 10 min) and was stored at -80°C until analysis.

Determination of zinc concentration

All zinc analyses were determined using glassware that had been washed in 30% nitric acid and rinsed with deionized distilled water. Tissue samples were prepared for analysis by wet digestion with HNO_3 and $HClO_4$ (Hill et al., 1983). Serum was prepared for analysis by diluting 1.0 ml of serum with 4.0 ml of deionized water. The dried, fat-free phalanges were ashed at 550°C for 48 h, and bone samples were then dissolved in 5 ml of 6 N HCl and diluted appropriately for Zn analysis (Wedekind et al., 1994). Zinc concentrations were determined by flame atomic absorption spectophotometry (novAA 400, Analytik Jena AG).

Lymphocyte proliferation

Lymphocyte proliferation was measured by using a colorimetric test with 3-(4,5-dimethyl-2-thiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) (Sigma Chemical Inc., St. Louis, USA) in cultures of purified peripheral blood mononuclear cells according to the method of Liu et al. (2003) and Mao et al. (2005). Briefly, mononuclear cells were isolated by gradient centrifugation from peripheral blood. The cells were washed three times in RPMI-1640 culture medium supplemented with 10% (vol/vol) heat-inactivated fetal calf serum, 100 U/ml of penicillin, 100 μg/ml of streptomycin and 25 mM N-(2-hydroxyethyl)-piperazine-N'-2-ethane-sulfonic acid. Following a final wash, cell activity was detected by trypan blue dye exclusion, the cells were counted, and the cell density was adjusted to 2×10^6 cells/ml culture medium. After that, the cells were cultured in 96-well microtiter plates with a total culture volume of 200 μl. Lymphocyte mitogen concanavalin A (ConA; Type. IV.C-2010, Sigma Chemical Inc., St. Louis, USA) was added at a final concentration of 16 μg/ml culture medium, and then the plates were incubated at 37°C in a 5% CO_2 incubator for 66 h. Subsequently, 10 μl of MTT solution (5 mg MTT/ml in 1/15 M phosphate-buffered saline, pH 7.6) was added to each well and the plates were incubated at 37°C for another 6 h. Following incubation, 100 μl of a 10% sodium dodecyl sulfate in 0.04 M HCl solution was added to lyse the cells and solubilize the MTT crystals. Finally, the plates were read via an automated ELISA reader (Bio-Rad, Model 680, Hercules, CA) at 570 nm.

Bovine serum albumin antibody analysis

Antibody response against bovine serum albumin was measured using ELISA according to a previously described method (Liu et al., 2003). Briefly, 96-well microtiter plates were coated with 100 μl of a solution containing 40 μg bovine serum albumin in 1ml of carbonate buffer (0.06 M, pH 9.6) and left overnight at 4°C. Plates were then washed four times with 0.01 M phosphate-buffered saline (pH 7.2) containing 0.05% Tween20 (Sigma Chemical). Serum samples were diluted with 0.01 M phosphate-buffered saline (pH 7.2) containing 10% horse serum at a dilution of 1:40. The diluted serum samples were added to the plates and incubated at 37°C for 1 h. Plates were then washed four times with 0.01 M phosphate-buffered saline (pH 7.2) containing 0.05% Tween 20. After washing, a 100 μl solution of rabbit anti-swine immunoglobulin G conjugated to horseradish peroxidase (Sigma Chemical) was added to each well. After incubation at 37°C for 1 h, the plates were washed and 100 μl of substrate, which contained 10 ml of citric acid buffer (0.05 M, pH 4.0), 100 μl of 27 mM 2,2'-azino-bis-(3-ethyl-benzthiazoline-6-sulfonic acid and 40 μl of 1% H_2O_2 was added to the wells. After incubation for 15 min at room temperature, the plates were read at an absorbance of 405 nm using an automated microplate reader (Bio-Rad, Model 680, Hercules, CA).

Serum interleukin-1 (IL-1) and interleukin-2 (IL-2)

Serum interleukin-1 and interleukin-2 concentrations were analyzed using commercially available swine interleukin-1 and interleukin-2 ELISA kit (Adlitteram Diagnostic Laboratories, Inc). The minimum detectable dose was 1.0 pg/ml for IL-1 and IL-2.

Statistical analysis

Data were analyzed by ANOVA using the GLM procedures of SAS (SAS Inst. Inc., Cary, NC) appropriate for a factorial arrangement of treatments in a randomized complete block design. The statistical model included the effects of LPS challenge diet, and their interactions. The analyses for the performance data were based on pen replication, and for the analyses of the other measurements, whereas individual pigs were considered as experimental units. A level of $p<0.05$ was used as the criterion for statistical significance, whereas a level of $p<0.10$ was taken to indicate a statistical trend.

RESULTS

In Exp. 1, the effect of dietary zinc level on performance of weanling pigs was presented in Table 2. Compared with pigs fed the basal diet, Zinc supplementation did not effect on growth performance in the whole trial (from d 0 to 21). Concentrations of Zn in tissues and serum were not affected by dietary treatment (Table 3).

Lymphocyte proliferation was not affected by

Table 2. Effects of supplemental Zn on growth performance of weanling pigs [a]

	Zn added (mg/kg)			SEM	p-value
	0	60	120		
ADG (g/d)					
0-7 d	169	182	168	14	0.74
7-14 d	226	220	216	14	0.88
14-21 d	280	285	283	25	0.89
0-21 d	225	235	226	13	0.83
ADFI (g/d)					
0-7 d	227	228	218	17	0.53
7-14 d	367	342	367	15	0.47
14-21 d	410	425	425	19	0.83
0-21 d	334	332	337	11	0.75
FCR					
0-7 d	1.35	1.27	1.33	0.06	0.69
7-14 d	1.63	1.57	1.73	0.06	0.35
14-21 d	1.48	1.49	1.49	0.08	0.87
0-21 d	1.48	1.41	1.49	0.05	0.29

[a] Values are means (n = 24) for six pens per treatment with four pigs per pen.
ADG = Average daily growth; ADFI = Average daily feed intake; FCR = Feed conversion ratio.

Table 3. Effects of supplemental Zn on tissue and serum Zn concentration of weanling pigs[a]

	Zn added (mg/kg)			SEM	p-value
	0	60	120		
Liver (mg/kg)	48.02	55.32	51.07	4.94	0.57
Kidney (mg/kg)	20.01	22.28	23.06	1.71	0.57
Spleen (mg/kg)	21.06	21.54	21.83	1.51	0.59
Phalanges (mg/kg)	72.80	80.47	81.39	8.07	0.72
Serum (μg/ml)	0.87	0.86	0.98	0.08	0.56

[a] Values are means (n = 6) for six pens per treatment with one pig per pen.

supplementation Zn (Table 4). The antibody response to BSA was reduced in pigs fed 120 mg/kg Zn ($p<0.05$) on d 7, compared to pigs fed the basal diet, but not on d 14.

In Exp. 2, performance data are presented in Table 5. Prior to LPS injection (from d 0 to 7), dietary treatment did not effect on growth performance. During the first week (from d 7 to 14) after the LPS injection, LPS challenge reduced ADG ($p<0.01$) by 25.6% and ADFI ($p<0.01$) by 18.2% compared to the saline-treated pigs, but FCR was not affected. Correspondingly, supplementation of Zn did not effect on ADFI and FCR, whereas ADG tended ($p<0.10$) to be improved when diets were supplemented with Zn. During the second week (from d 14 to 21) after the LPS injection, there was neither LPS challenge nor diet effect on ADG, ADFI, and FCR. In the whole trial (from d 0 to 21), LPS challenge and supplementation of Zn had no effect on ADG, ADFI and FCR.

The results of lymphocyte proliferation are shown in Table 6. LPS challenge increased ($p<0.05$) blood lymphocyte proliferation when incubated with 16 μg/ml ConA. There was a trend for lymphocyte proliferation to be enhanced with supplemental Zn ($p<0.10$).

LPS challenge or diet has no effect on serum antibody response to BSA on d 7 and 12 after the injection of BSA (Table 6). There was no LPS challenge×diet interactions observed for serum IL-1 and IL-2 concentrations. Concentration of serum IL-1 was increased ($p<0.01$) 46.5% by LPS challenge at 3 h post-injection, but concentration of serum IL-2 was not affected. Dietary treatment had no effect on serum IL-1 and IL-2 concentrations.

Table 4. Effects of supplemental Zn on peripheral blood lymphocyte proliferation (PBLP) and antibody response to BSA [a]

	Zn added (mg/kg)			SEM	p-value
	0	60	120		
PBLP	0.305	0.321	0.364	0.037	0.526
BSA response, absorbance					
d 7	0.349[b]	0.328[bc]	0.224[c]	0.038	0.088
d 14	0.587	0.577	0.502	0.084	0.738

[a] Values are means (n = 6) for six pens per treatment with one pig per pen.
[b,c] Mean values in a row without the same superscript small letter are different ($p<0.05$).
PBLP = Lymphocyte proliferation; BSA = Bovine serum albumin.

Table 5. Effects of lipopolysaccharide (LPS) challenge and dietary zinc on growth performance of weanling pigs[a, b]

	-LPS			+LPS			SEM
	Zn added (mg/kg)			Zn added (mg/kg)			
	0	60	120	0	60	120	
ADG (g/d)							
0-7 d	343	355	357	370	362	376	33
7-14 d [cd]	428	415	426	297	312	332	42
14-21 d	442	445	476	425	435	454	42
0-21 d	412	428	426	363	371	369	26
ADFI (g/d)							
0-7 d	591	586	600	611	631	628	29
7-14 d [c]	678	673	664	523	558	597	30
14-21 d	782	819	804	799	791	821	42
0-21 d	703	693	691	657	661	675	35
FCR							
0-7 d	1.72	1.65	1.68	1.65	1.74	1.67	0.08
7-14 d	1.58	1.62	1.56	1.76	1.79	1.80	0.11
14-21 d	1.79	1.84	1.69	1.81	1.78	1.70	0.05
0-21 d	1.71	1.62	1.63	1.81	1.78	1.70	0.09

[a] Diets are the same as diets of Exp. 1.
[b] Lipopolysaccharide was injected on 7 d. Values are means (n = 9) for three pens per treatment with three pigs per pen.
[c] LPS effect ($p<0.01$). [d] Diet effect ($p<0.10$).

DISCUSSION

Zinc is an essential trace element involved in many metabolic functions. Zinc deficiency reduced growth, development and immune system function (Rink and Kirchner, 2000). The NRC (1998) Zn requirement for nursery pigs is set at 100 mg/kg and was based on the level of Zn needed to maximize growth. In our study, the Zn concentration in a corn-soybean meal diet did not impair growth performance. This finding is consistent with some previous studies (Jiang et al., 1987; Wedekind et al., 1994; Cheng et al., 1998; Spears et al., 2002; Lallès et al., 2007) that zinc supplementation did not enhance growth rate of nursery pigs. Pharmacological levels of Zn (e.g., levels of 300 to 3,000 mg/kg) are often used in the diets of nursery pigs immediately following weaning and have been reported to enhance growth performance (Hill et al., 2000; Case and Carlson, 2002). Hahn and Baker (1993) reported $ZnSO_4$ addition increased these performance indices only at the 3,000 mg of Zn/kg level of supplementation. Smith et al. (1961) indicated that swine fed semi-purified diets containing isolated soybean protein required at least 46 ppm zinc for maximum performance. In our study, the basal diet containing 36.75 mg/kg of Zn was close to the threshold of zinc level required to maintain normal growth of the weanling pigs, and supplementation of zinc were much lower than those pharmacological levels. Therefore, dietary zinc levels did not affect growth performance. The results of this experiment indicate that the current NRC (1998) recommendations for Zn (100 mg/kg for pigs between 5 and 10 kg of BW and 80 mg/kg for pigs between 10 and 20 kg of BW) were sufficient to maximize growth performance in weanling pigs.

Tissue concentrations of Zn have been demonstrated to be responsive to supplementation of Zn in pigs fed Zn-deficient diets (17 mg/kg of Zn) (Swinkels et al., 1996) and

Table 6. Effects of lipopolysaccharide (LPS) challenge and dietary zinc on peripheral blood lymphocyte proliferation (PBLP), antibody response to BSA, IL-1and IL-2 levels [a, b]

	-LPS			+LPS			SEM
	Zn added (mg/kg)			Zn added (mg/kg)			
	0	60	120	0	60	120	
PBLP [cd]	0.366	0.402	0.455	0.431	0.535	0.560	0.042
BSA response, absorbance							
d 7	0.401	0.355	0.390	0.374	0.371	0.353	0.048
d 12	0.654	0.683	0.638	0.615	0.630	0.662	0.055
IL-1 (pg/ml)[e]	73	79	75	127	150	148	15
IL-2 (pg/ml)	47	52	56	51	63	60	8

[a] Diets are the same as diets of Exp. 1.
[b] Lipopolysaccharide was injected on 7 d. Values are means (n = 6) for six pigs (one pig per pen).
[c] LPS effect ($p<0.05$). [d] Diet effect ($p<0.10$). [e] LPS effect ($p<0.01$).
PBLP = Lymphocyte proliferation; BSA = Bovine serum albumin; IL-1 = Interleukin-1; IL-2 = Interleukin-2.

when diets were supplemented with pharmacological levels of Zn (Schell and Kornegay, 1996; Case and Carlson, 2002). In the present study, dietary zinc meet the normal physiological requirements of weanling pigs. Therefore, tissue and serum Zn concentrations were not affected.

A variety of *in vivo* and *in vitro* effects of zinc on immune cells mainly depend on the zinc concentration (Ibs and Rink, 2003). Zinc deficiency as well as supraphysiologic levels impaired immune function, yet there was no consistent effect of Zn supplementation on immune response of pig. Hall et al. (1993) reported reduced proliferative response to PWM in lymphocytes from pigs receiving no supplemental Zn compared with pigs supplemented with 40 mg/kg of Zn. However, van Heugten et al. (2003) observed that lymphocyte proliferation was not affected by supplemental Zn as $ZnSO_4$ when PHA was used as the mitogen. In the present study, lymphocyte proliferation was not affected by supplementation Zn. The unchanged tissue Zn and serum Zn may in part explain the unchanged lymphocyte proliferation. In addition, the differences in responses observed also may be caused by mitogens. Cheng et al. (1998) reported that humoral immune responses of pigs to sheep red blood cells and ovalbumin were not affected by supplementation of 100 mg/kg Zn as $ZnSO_4$ to a basal diet containing 32 mg/kg of Zn. van Heugten et al. (2003) reported that supplementation of 80 mg/kg of Zn and 160 mg/kg of Zn as $ZnSO_4$ reduced the primary antibody response to ovalbumin on d 7, compared to control weanling pigs, but not on d 14. However, antibody response to SRBC was not affected by dietary treatments. In our study, supplementation of 120 mg/kg Zn reduced the primary antibody response to BSA on d 7 compared with basal diet, but not on d 14.

An immunological challenge results in reduced feed intake, lean muscle accretion and growth. It has been well documented that these changes observed in animals subjected to an immunological challenge are mediated by pro-inflammatory cytokines (Johnson, 1997; Jacobi et al., 2006). During the challenge, one of the first responses of an animal is to release pro-inflammatory cytokines such as IL-1β and TNF-α from macrophages (Spurlock, 1997). Furthermore, Hellerstein et al. (1989) have shown that anorexia of rats was induced by interleukin-1. Lipopolysaccharide is a molecule found in the membrane of all gram-negative bacteria. LPS induces symptoms of acute bacterial infection, including anorexia, hypersomnia, and fever in weaned pigs. The effects of LPS are due to its ability to stimulate macrophages to synthesize and secrete pro-inflammatory cytokines (Johnson and von Borell, 1994). In the current study, LPS challenge significantly decreased average daily gain and average daily feed intake of weanling pigs during d 7 to 14 after LPS challenge. This finding is consistent with some previous studies in pigs (Johnson, 1997; Liu et al., 2003; Mao et al., 2005). However, average daily gain and average daily feed intake were not affected during d 14 to 21 after LPS challenge, which agrees with the results of Balaji et al. (2000) who reported that LPS induces a short duration response rather than a chronic immunological stress. In addition, Liu et al. (2008) found that LPS challenge severely decreased performance of weaned pigs during 48 h post-challenge.

In the present study, LPS injection also resulted in an increased lymphocyte proliferation and concentration of IL-1. It has been shown that the increased lymphocyte proliferation and IL-1 concentration indicated an activation of the immune system. The result of this process is that nutrients are directed away from tissue growth to support immune function (Spurlock, 1997), which will decrease the efficiency of nutrient utilization for growth. Groote et al. (1992) reported that cytokines IL-1β, TNF-α and IL-6 are preferentially stimulated by LPS whereas IL-2, IFN-gamma and GM-CSF are stimulated by PHA. This might partially explain why serum interleukin-2 concentration was not affected by the LPS challenge in the current experiment. Humoral immune response of pigs was not affected by the LPS challenge, and this finding is consistent with some previous studies in pigs (van Heugten et al., 1994; Kegley et al., 2001; Guo et al., 2008).

Until now, few researches were conducted to evaluate the effect of zinc on growth performance of pigs during an immunological challenge. In our study, dietary treatment had no effect on pig performance before LPS challenge. Zn supplementation did not alleviate daily feed intake depression during d 7 to 14 after LPS challenge, but there was a trend for average daily gain of pig to be enhanced with supplemental Zn. This indicated that Zn supplementation may alter the negative effects of an immunological stress. Studies reveal that lymphocytes proliferation is increased after zinc supplementation. Furthermore, the release of cytokines such as IL-1 and -6, TNF-α is induced when peripheral blood mononuclear cells are incubated with zinc *in vitro*. Zinc supplementation also results in elevated production of IL-2. In the present study, there was a trend for lymphocyte proliferation to be enhanced with supplemental Zn, but IL-1 and IL-2 concentrations were not affected *in vivo*. Furthermore, we found humoral immune response of immunologically challenged pigs was not affected by supplementation of Zn.

IMPLICATIONS

In the present study, we demonstrated that the level of Zn recommended by NRC (1998) for weanling pigs was sufficient for optimal growth performance and immune responses. A corn-soybean meal basal diet contained 36.75 mg/kg total Zn may be adequate to sustain overall immunity

in normal pigs for a short-term period. LPS challenge reduced average daily gain and average daily feed intake of pigs. Zn supplementation had a tendency to increase lymphocyte proliferation of immunologically challenged pigs. Daily gain tended to be improved when diets were supplemented with Zn, which indicated that Zn requirements may be higher for pigs experiencing an acute phase response than for healthy pigs. In this study, it is still not clear whether Zn can be of benefit in immunologically challenged pigs, the effects of Zn on immune system function and growth under immunological stress conditions is further studied.

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