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Effect of Dietary β-1,3/1,6-glucan Supplementation on Growth Performance, Immune Response and Plasma Prostaglandin E_2, Growth Hormone and Ghrelin in Weanling Piglets

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ABSTRACT : The experiment was conducted to evaluate the effect of β-1,3/1,6-glucan on growth performance, immunity and endocrine responses of weanling piglets. One hundred and eighty weanling piglets (Landrace×Large White, 7.20±0.25 kg BW and 28±2 d of age) were randomly fed 1 of 5 treatment diets containing dietary β-1,3/1,6-glucan supplemented at 0, 25, 50, 100 and 200 mg/kg for 4 wks. Each treatment was replicated in 6 pens containing 6 pigs per pen. On d 14 and 28, body weight gain, feed consumption and feed efficiency were recorded as measures of growth performance. Peripheral blood lymphocyte proliferation and serum immunoglobulin G (IgG) were measured to study the effect of dietary β-1,3/1,6-glucan supplementation on immune function. Plasma prostaglandin E_2 (PGE_2), growth hormone (GH) and ghrelin were measured to investigate endocrine response to β-1,3/1,6-glucan supplementation. Our results suggest that average daily gain (ADG) and feed efficiency had a quadratic increase trend with dietary β-1,3/1,6-glucan supplementation from d 14 to 28, whereas it had no significant effect on average daily feed intake (ADFI). The treatment group fed with 50 mg/kg dietary β-1,3/1,6-glucan supplementation showed a numerical increase in ghrelin, a similar change trend with ADG and no significant effect on GH. Lymphocyte proliferation indices, serum IgG and plasma PGE_2 concentrations varied linearly with dietary supplementation levels of β-1,3/1,6-glucan on d 14. Higher levels of β-1,3/1,6-glucan may have a transient immuno-enhancing effect on the cellular and humoral immune function of weanling piglets via decreased PGE_2. Taking into account both immune response and growth performance, the most suitable dietary supplementation level of β-1,3/1,6-glucan is 50 mg/kg for weanling piglets. (**Key Words :** β-1,3/1,6-glucan, Growth Performance, Immune Response, PGE_2, Ghrelin, Weanling Piglets)

INTRODUCTION

Using antibiotics as a dietary supplement can improve growth performance and feed efficiency in domestic animals and poultry. However, long-term and nonspecific application of antibiotics may not only lead to the reduced function of the immune system, but also result in bacterial resistance to antibiotics. Furthermore, there is increasing concern that residues of antibiotics may be hazardous to the safety of animal products and human health. The search for alternatives to the feeding of antibiotics in animal nutrition is an ongoing problem. Using immunomodulators to alter the immune function of animals is considered a potential means for improving growth performance and health status. β-1,3/1,6-glucan, derived from the cell wall of *Saccharomyces cerevisiae*, is a polyglucose consisting of a linear backbone of β-1,3-linked D-glucopyranosyl units with degrees of branching at the C-6 position (Jorgensen and Robertsen, 1995). β-1,3/1,6-glucan is known to possess antitumor (Ohno et al., 1987) and antimicrobial properties (Robertsen et al., 1990; Hetland et al., 2000; Ortuno et al., 2002; Lowry et al., 2005; Chae et al., 2006; Huff et al., 2006) that enhance host immune function. In pigs, the stimulatory effects of β-glucan on specific and non-specific immune responses have been demonstrated (Mowat, 1987; Stokes et al., 1987; Dritz et al., 1995; Mao et al., 2005; Eicher et al., 2006; Hahn et al., 2006; Li et al., 2006), and shown to have beneficial effects on growth performance (Schoenherr et al., 1994; Dritz et al., 1995; Eicher et al., 2006; Hahn et al., 2006; Li et al., 2006). However, one of the problems is the lack of information about the mechanisms behind β-1,3/1,6-glucan activity in piglets. Therefore, the aim is to explore the potential action

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Table 1. Ingredient and chemical composition of the basal diets for weanling piglets (%, as fed basis)

Ingredients (%)	Composition
Corn[1]	54.7
Soybean meal (45% CP)	26
Fish meal (64% CP)	5
Whey powder[2]	6
Soybean oil	4
Dicalcium phosphate	0.65
Limestone	1.00
Salt	0.35
L-lysine HCl	0.25
DL-methionine	0.05
Acidifying agents	0.5
Vitamin and trace mineral premix[3]	1.00
Total	100.0
Calculated nutrient composition[4] (%)	
DE (MJ/kg)	14.20
Crude protein	20.0
Lysine	1.46
Methionine	0.40
Calcium	0.85
Phosphorus	0.68

[1] β-1,3/1,6-glucan replaced corn in the experimental treatments.
[2] Whey powder was a product of Calva Product Inc., Acampo, C. A.
[3] Premix supplied the following per kilogram of diet: Vitamin A , 15,000 IU; Vitamin D_3, 3,000 IU; Vitamin E, 40 IU; Vitamin K_3, 3.5 mg; Vitamin B_1, 4 mg; Vitamin B_2, 20 mg; Vitamin B_6, 4.5 mg; Vitamin B_{12}, 0.015 mg; nicotinic acid, 45 mg; pantothenic acid, 15 mg; folic acid, 0.7 mg; Biotin, 0.13 mg; Choline Chloride, 500 g; Fe, 150 mg; Cu, 275 mg; Zn, 230 mg; Mn, 55 mg; I, 0.80 mg; Se, 0.38 mg.
[4] Calculated based on the feed ingredient composition data from our lab and from manufacturers value.

mechanisms of β-1,3/1,6-glucan by evaluating the effects of dietary supplementation on the performance, immunity and endocrine responses of weanling piglets. In addition, the experimental diets were supplemented with varying levels of β-1,3/1,6-glucan to determine the most effective supplementation rate.

MATERIALS AND METHODS

Animals and experimental design

A total of 180 weanling piglets (Landrace×Large White; 7.20±0.25 kg BW and 28±2 d of age) were randomly allocated to five dietary treatment groups. Each treatment group had 6 replicates with 6 piglets per pen. Piglets were reared in an isolated, disinfected and clean experimental house with slotted floor pens. Each pen contained a self-feeder and a nipple drinker to allow *ad libitum* access to feed and water. The room temperature was controlled (25 to 28°C). The experimental diets (Table 1) were formulated to meet the National Academy of Science-National Research Council (NAS-NRC, 1998) requirements for all nutrients. The experimental diets were formulated using corn, soybean meal, fish meal, whey powder, fatty powder and complex vitamin and mineral supplements. A basal diet was formulated, and the remaining four diets were supplemented with 25, 50, 100, 200 mg/kg of β-1,3/1,6-glucan respectively. The experimental diets were fed to piglets for 4 wks. β-1,3/1,6-glucan was extracted from *Saccharomyces cerevisiae* in our laboratory according to the method of Hunter et al. (2002) with some modifications. The chemical content of the β-1,3/1,6-glucan product is: 91.5% glucan, 1.15% crude protein, 0.43% crude fat and other unknown materials (Zhang et al., 2008). Infra-red (IR) and nuclear magnetic resonance (NMR) analysis was used to confirm that the β-glucan product contained a primary chain consisting of β-1,3-linkages, and also to determine the degree of β-1,6-linkages in the side chain. The polymer unit is listed below:

-[-β-D-Glcp-(1,3)- β-D-Glcp-(1,3)- β-D-Glcp-(1,3)- β-D-GlcpNAc-]
| 1,6
-1,6-β-D-Glcp-(1,6)- β-D-Quip-(1,6)

Its average molecular weight was determined by light-scattering to be about 190 kD The β-1,3/1,6-glucan used had no effect on endotoxin production as shown by the measurement of plasma endotoxin using the commercial endotoxin quantitative kit (Limulus amebocyte lysate, Pyrochromer quantitative kit, c0060, ACC, Inc. USA).

Data and sample collection

Piglets were weighed and feed consumption was measured on d 14 and 28 to calculate ADG, ADFI and feed efficiency (gain/feed) for each pen. Peripheral blood was collected into 10 ml LH Lithium heparinized vacuum tubes (Greiner bio-one GmbH, Austria) for determination of lymphocyte proliferation, plasma PGE_2, GH and ghrelin concentrations. On d 14 and 28, 3 ml of blood was collected into plain vacuum tubes via anterior vena cava venapuncture to determine serum IgG levels. Blood samples were centrifuged at 3,000×g for 10 min at 4°C, and the supernatant obtained was stored at -80°C.

Lymphocyte proliferation

Lymphocyte proliferation was measured using a colorimetric assay with 3-(4,5-dimethlthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT, M-2128, Sigma Chemical Inc.) in cultures of purified peripheral blood mononuclear cells, according to the method of Mosmann (1983) with some modifications. Briefly, peripheral blood mononuclear cells were separated on a Ficoll-sodium diatrizoate gradient with a specific density of 1.077 g/ml (Academy of Military Medical Science, China) in sterile polyethylene tubes, and centrifuged at 2,500×g for 20 min

Table 2. Effect of β-1,3/1,6-glucan on the growth performance of weanling piglets[1]

Item	β-1,3/1,6-glucan (mg/kg)					SEM[3]	p-value[2]	
	0	25	50	100	200		Linear	Quadratic
ADG (g/d)								
d 0 to 14	265	221	266	235	221	11	0.542	0.992
d 14 to 28	537	606	650	610	569	18	0.335	0.089
d 0 to 28	401	414	458	423	395	13	0.627	0.267
ADFI (g/d)								
d 0 to 14	389	330	353	336	329	17	0.806	0.625
d 14 to 28	930	893	951	921	990	23	0.385	0.528
d 0 to 28	660	611	652	629	660	16	0.790	0.535
Gain/feed (g/g)								
d 0 to 14	0.68	0.67	0.75	0.70	0.67	0.02	0.596	0.445
d 14 to 28	0.58	0.68	0.68	0.66	0.57	0.03	0.115	0.026
d 0 to 28	0.61	0.68	0.70	0.67	0.60	0.02	0.165	0.036
Diarrhea rate[4] (%)	1.82	1.97	1.83	2.63	3.07	0.001	0.907	0.420
Death rate[5] (%)	0.47	0.93	0.47	0.47	0.47	0.002	0.947	0.803

[1] Values are means of 6 pens of pigs (n = 6 pigs per pen).
[2] p-value of a linear (L) or Quadratic (Q) effect of dietary treatment.
[3] Standard error of the mean. [4] Values are mean of d 0 to 28 diarrhea rate. [5] Values are mean of d 0 to 28 death rate.

at room temperature. The cells were then washed with 3 ml RPMI-1640 solution three times, centrifuged at 2,000×g for 10 min at room temperature, and resuspended in RPMI-1640 medium with 1% HEPES buffer containing 10% fetal calf serum, 100 U/ml penicillin, and 0.1 mg/ml streptomycin (pH 7.2) to a concentration of 1-2×10^6 cells/ml. Cell viability was assessed by trypan blue exclusion and was always high (>95%). One hundred and ninety microlitres of cell suspension per well was distributed in 96 well culture plates (Costar, USA). Lipopolysaccharide (LPS, Sigma) at 16 μg/ml of final concentration was added to wells for stimulating B lymphocyte proliferation, and concanavalin A (Con A, Sigma) at 15 μg/ml was added to stimulate T lymphocyte proliferation. Wells without mitogen were used as controls. All the tests were carried out in triplicate. After 68 h incubation in a 5% CO_2 incubator at 37°C, MTT solution was added to all wells to a final concentration of 5 μg/ml, and the cells were incubated at 37°C for another 4 h. After 4h, 100 μl of fresh 10% SDS-HCL was added to each well and mixed. After 30 min, the plates were read on a micro-reader at 570 nm. Stimulation indices (SI) were calculated based on the following formula: SI = (Absorbance value for mitogen-stimulated cultures)/(Absorbance value for non-stimulated cultures).

Measurement of serum IgG

The concentration of serum IgG was measured using a commercial kit (pig ELISA quantitation kit; Benthyl Laboratories, Montgomery, TX, USA), according to manufacturer's instructions. Serum was diluted 1:100,000 with assay buffer for analysis of IgG.

Assessment of hormones

Plasma PGE_2, GH concentrations were measured using commercially available radioimmunoassay kits (Beijing Sino-UK Institute of Biological Technology, Beijing, China). The sensitivity (minimum detectable concentration) was 6.25 pg/ml for porcine plasma prostaglandin E_2, and 0.1 pg/ml for GH, with an intraassay CV of <10% for both PGE_2 and GH. Plasma ghrelin was quantified using a commercially available ghrelin radioimmunoassay kit (Phoenix Pharmaceutical Inc., Belmont, CA, USA). Mean interassay and intraassay CV was less than 13% and assay sensitivity was 0.1 pg/ml.

Statistical analysis

All data were analyzed using SPSS 10.0 for Windows. Dose effects of dietary β-1,3/1,6-glucan on pig growth performance, immunity and PGE_2 were assessed by polynomial contrasts (linear and quadratic). Plasma ghrelin and GH concentrations were analyzed by ANOVA with time repeated measurements. The statistical model for ghrelin and GH consisted of dietary treatment, time, treatment and time interactions. Pens were used as the experimental unit for all analyses. Effects were considered significant at $p<0.05$, and probability values between $p>0.05$ and $p<0.10$ were considered trends.

RESULTS

Growth performance

Growth performance data are shown in Table 2. From d 0 to 14, no significant differences ($p>0.05$) in ADG, ADFI, gain/feed ratio, diarrhea rate and death rate were observed among dietary treatments. From d 14 to 28, there was a quadratic increase for gain/feed ratio ($p = 0.026$) and a quadratic increase trend for ADG ($p = 0.089$) with the level of dietary β-1,3/1,6-glucan supplementation. Piglets

Table 3. Effect of β-1,3/1,6-glucan on immune responses of weanling piglets[1]

Item	β-1,3/1,6-glucan (mg/kg)					SEM[3]	p-value[2]	
	0	25	50	100	200		Linear	Quadratic
d 14								
ConA	1.04[c]	1.17[bc]	1.31[ab]	1.35[ab]	1.52[a]	0.05	0.003	0.230
LPS	1.04[b]	1.32[a]	1.33[a]	1.36[a]	1.38[a]	0.03	0.005	0.000
IgG (mg/ml)	5.47[b]	6.00[ab]	6.36[ab]	6.57[ab]	7.47[a]	0.27	0.032	0.840
PGE_2 (pg/ml)	133.50[a]	111.32[ab]	113.18[ab]	80.77[ab]	70.90[b]	8.75	0.024	0.837
d 28								
ConA	1.19	1.11	1.20	1.20	1.15	0.03	0.771	0.860
LPS	1.02	1.16	1.06	1.03	1.14	0.03	0.418	0.596
IgG (mg/ml)	6.83	6.70	6.63	7.81	7.69	0.40	0.844	0.818
PGE_2 (pg/ml)	117.66	119.75	91.92	95.90	81.35	10.73	0.785	0.907

[a, b ,c] Values with different subscripts in the same row differ significantly, $p<0.05$.
[1] Each mean value represents 6 pens with 1 pig per pen.
[2] p-value of a linear (L) or Quadratic (Q) effect of dietary treatment. [3] Standard error of the mean.

Table 4. Effect of β-1,3/1,6-glucan on plasma ghrelin and GH concentration of weanling piglets[1]

Item	β-1,3/1,6-glucan (mg/kg)				SEM[3]	p-value[2]		
	0		50					
	d 14	d 28	d 14	d 28		Dose	Time	Interaction
GH (ng/ml)	10.94	12.34	11.33	13.65	0.55	0.143	0.005	0.413
Ghrelin (pg/ml)	60.91	60.13	61.10	68.87	3.35	0.216	0.330	0.236

[1] Each mean value represents 6 pens with 2 pig per pen. [2] p-value of dose, time and dose×time. [3] Standard error of the mean.

supplemented with 50 mg/kg of β-1,3/1,6-glucan had greater ADG and feed efficiency compared with other treatment groups. The gain/feed ratio of piglets from d 0 to 28 tended to respond to β-1,3/1,6-glucan supplementation in a quadratic fashion ($p = 0.036$). No effect of β-1,3/1,6-glucan on ADFI was found.

Immune responses

Table 3 showed that on d 14, the lymphocyte proliferation responses to ConA and LPS increased linearly ($p<0.01$) with increasing β-1,3/1,6-glucan supplementation. The responses generally were pronounced in piglets fed 200 mg/kg of β-1,3/1,6-glucan. However, on d 28, no significant differences were found in lymphocyte proliferation among treatments. On d 14, there was a linear increase ($p<0.10$) for serum IgG concentrations, and piglets fed 200 mg/kg β-1,3/1,6-glucan had greater serum IgG concentration ($p<0.01$) compared with the other treatments. However, on d 28, no statistically significant linear and quadratic effect of dietary treatment was observed for serum IgG.

Endocrine responses

Table 3 showed that there was a linear decrease ($p<0.05$) for plasma PGE_2 on d 14 but no effect on d 28. Plasma ghrelin (Table 4) was unaffected by dietary treatment ($p = 0.216$) or time ($p = 0.330$), Whereas, piglets fed 50 mg/kg β-1,3/1,6-glucan had a numerical increase in ghrelin concentrations. There was no treatment×time interaction ($p = 0.236$) for plasma ghrelin. Although there was no treatment×time interaction ($p = 0.413$), there was still an overall time effect ($p<0.01$) on GH. Serum GH on d 28 was significantly higher ($p = 0.005$) than that on d 14.

DISCUSSION

Growth performance

We observed that ADFI was not significantly influenced by dietary β-1,3/1,6-glucan supplementation. Dietary β-1,3/1,6-glucan supplementation had a quadratic increase trend for ADG and gain/feed ratio from d 14 to 28 and d 0 to 28. Piglets fed 50 mg/kg β-1,3/1,6-glucan had higher ADG and greater feed efficiency during d 14 to 28 and d 0 to 28 compared with other treatments. The overall trend of ADG and gain/feed ratio improvement with β-glucan supplementation was similar to that reported by Schoenherr et al. (1994) and Dritz et al. (1995). Schoenherr et al. (1994) evaluated growth performance of weanling piglets fed 0, 250, 500, 750, 1,000, and 1,250 mg/kg β-glucan (MacroGard). Although no improvements in growth were observed in the first 2 wks, β-glucan improved overall (d 0 to 34 after weaning) ADG and feed efficiency. Schoenherr et al. (1994) also concluded that the optimal supplementation level of β-glucan is between 250 mg/kg and 500 mg/kg when fed throughout the nursery period, and that a supplementation rate of β-glucan higher than 1,000 mg/kg resulted in decreased growth. Dritz et al. (1995) also reported improved ADG when piglets were fed 250 mg/kg β-glucan (MacroGard-S, Provesta Corp., Bartlesville, OK) for 28 d. In contrast, some studies reported that the addition of β-glucan to diets only increased ADG, and had no

significant effect on ADFI and feed efficiency (Eicher et al., 2006; Hahn et al., 2006; Li et al., 2006). Hahn et al. (2006) reported that, although no significant differences in ADFI and gain/feed ratio were observed between dietary β-glucan (Glucagen, Enbiotec Company, Seoul, Korea) treatments, there was a linear increase trend in ADG as the dietary β-glucan concentration (0, 0.01, 0.02, 0.03, and 0.04%) increased during phase II (3 to 5 wk). Eicher et al. (2006) also reported that yeast cell wall β-glucan (Energy Plus, Natural Chem Industries, LTD, Houston, TX) increased ADG significantly. Likewise, Li et al. (2006) reported that the addition of β-glucan (containing 86.1% β-glucan, 4.19% protein, 1.23% lipids and unknown materials) to weaned pig diets had no effect on ADFI and gain/feed ratio in any period, except had a quadratic affect on ADG between d 14 to 28 and d 0 to 28. Furthermore, Li et al. (2006) reported that the optimal concentration of yeast β-glucan for improving growth was 50 mg/kg when fed throughout the nursery period. However, Hiss and Sauerwein (2003) reported that the continuous supplementation of weanling pig diets with 150 or 300 mg/kg of a yeast-derived β-glucan product resulted in a numerical increase in ADG because of increased feed intake.

We observed that for improvement in ADG, the optimal supplementation of β-1,3/1,6-glucan was 50 mg/kg, much less than previously reported. Differences in dosage amongst various studies might be attributed to the influence of purity of β-glucan used. Studies on structure-function relationships of β-glucan have revealed that molecular weight, conformation and degree of branching may affect the binding of β-glucan with its receptors and therefore influence its activities (Bohn et al., 1995; Brown et al., 2003).

Except for the 50 mg/kg β-1,3/1,6-glucan treatment group, we also observed that dietary β-1,3/1,6-glucan supplementation reduced ADG from d 0 to 14, whereas from d 14 to 28, there was trend for an increased ADG. A possible reason for this is that from d 0 to 14, excessive immune responses were induced by the higher level of β-1,3/1,6-glucan. As a result, a slightly increased diarrhea rate and a slightly reduced ADFI were found, indicating that nutrients may have been repartitioned toward the immune system and not growth (Klasing et al., 1987; Dritz et al., 1995). Continuous β-1,3/1,6-glucan addition resulted in increased tolerance to oral antigen (Dritz et al., 1995). This could explain the increased ADG for pigs fed diets supplemented with β-1,3/1,6-glucan compared with pigs fed the control diet.

Immune responses

Lymphocyte function can be ascertained by assessing proliferation ability induced by T cell mitogens, ConA and PHA as well as T cell-dependent B cell mitogens, LPS and PWM. Our results found that β-1,3/1,6-glucan has transient effects on lymphocyte proliferation. In agreement with our findings, Suzuki et al. (1989) showed that the proliferative responses of spleen cells from β-glucan administered mice T-cell and B-cell mitogens were greater than those from normal mice. Likewise, some previous reports have also demonstrated that β-glucan can directly influence the activity of immune cells, including T and B cells (Cantrell et al., 1984; Hashimoto et al., 1991; Yun, 2003). However, our studies show that continuous stimulation with β-1,3/1,6-glucan had no significant effect on lymphocyte proliferation on d 28. Similarly, Hiss and Sauerwein (2003) reported that continuous β-1,3/1,6-glucan treatment had no effect on lymphocyte proliferation in weaned piglets. Moreover, Cheng et al. (2004) also found that supplementation of β-glucan did not elevate the lymphocyte blastogensis following stimulation with different mitogens. Castro et al. (1999) reported that high doses of β-1,3/1,6-glucan could stimulate the respiratory burst of fish leukocytes *in vitro*, but after sometime, the cells did not respond to a second stimulation with β-1,3/1,6-glucan The reason might be that the immune cells became exhausted and did not respond to a longer period of stimulation with β-1,3/1,6-glucan. These results also indicate that the immunomodulatory properties of β-1,3/1,6-glucan are associated with administration period.

Total serum IgG concentrations were measured to assess the effects of dietary β-1,3/1,6-glucan supplementation on nonspecific humoral immune competences in weanling piglets. It has been reported that maternal IgG reaches minimal levels in a piglet's system by 4 weeks of age (Hunter, 1986). Furthermore, active synthesis of IgG does not begin until 5 weeks of age (Hunter, 1986), with increases once again starting at 7 weeks of age (Bianchi et al., 1995). Low IgG concentrations in weanling piglets at 4 to 8 weeks of age may be associated with decreased immunity and increased susceptibility to disease. We observed that oral supplementation with β-1,3/1,6-glucan resulted in temporarily increased serum IgG concentrations of weanling piglets. Previous studies also showed that serum or colostral IgG levels could be affected by immunomodulator β-1,3/1,6-glucan (Krakowski et al., 1999, 2002; Zhang et al., 2008). Therefore, our results indicate that dietary β-1,3/1,6-glucan supplementation could enhance humoral immune function of weanling piglets.

Immune activation is associated with exterior antigen stimulation, for example, in vaccine inoculation, bacterial and viral infections or non-specific immunoregulator inducement. This study was carried out in an isolated and clean experimental house. Moreover, low diarrhea rate and death rate was observed during the experimental period.

Therefore, the increases in serum IgG and lymphocyte proliferation were mostly the result of stimulation by dietary β-1,3/1,6-glucan supplementation and not from infection. Based on these findings, our research indicates that β-1,3/1,6-glucan might potentiate cellular and humoral immune functions of weanling piglets in an environment with minimal disease challenges. However, the mechanisms underlying the relationship between β-1,3/1,6-glucan supplementation and immune response is still unclear. Hence, it is essential that further research is carried out on the immunomodulatory impact of β-1,3/1,6-glucan in models of infection.

Interestingly, although dietary supplementation with 200 mg/kg β-1,3/1,6-glucan might significantly increase lymphocyte proliferation and induce weanling piglets to produce higher levels of serum IgG than controls, ADG did not improved when compared with other treatments. Consistent with this, White et al. (2002) also found that IgG concentrations were enhanced by the addition of a yeast product in diets of weanling piglets, however, growth measures were unchanged. Huff et al. (2006) reported the immune stimulation provided by β-1,3/1,6-glucan might result in decreased production values for birds raised in an environment with minimal disease challenges. It is generally accepted that mounting immune responses likely requires using resources that could otherwise be allocated to other biological processes, for example, growth, reproduction or lactation (Klasing et al., 1987; Demas et al., 1997; Colditz, 2002). Klasing et al. (1987) reported that increased concentrations of IL-1 were associated with decreased feed intake and growth performance. Previous studies have demonstrated that high level of β-glucan induced the release of proinflammatory cytokines such as IL-1 and TNF-α from mononuclear phagocytes (Poutaiaka et al., 1993; Vetvicka et al., 2004). Moreover, we also observed that dietary supplementation with 200 mg/kg of β-1,3/1,6-glucan resulted in a significantly enhanced immune response, whereas 50 mg/kg of β-1,3/1,6-glucan supplementation resulted in a slight immune response. This could explain the decreased growth performance for pigs fed diets supplemented with 200 mg/kg of β-1,3/1,6-glucan compared with pigs fed diets supplemented with 50 mg/kg of β-1,3/1,6-glucan. Taking into account growth performance and immune response, the most suitable dietary supplementation level of β-1,3/1,6-glucan is 50 mg/kg for weanling piglets. This is similar to the level recorded for broilers (Zhang et al., 2008). Further investigation is required to determine the optimum supplementation level of β-1,3/1,6-glucan to achieve greatest performance.

Endocrine responses

Piglets fed β-1,3/1,6-glucan had lower concentration of PGE_2 concentration compared with those fed the control diet. In agreement with our findings, Castro et al. (1994) observed that the release of macrophage arachidonic acid metabolite (prostaglandin) in response to *Candida albicans* was inhibited to a significant, but lower degree by soluble β-glucan. Similarly, Mao et al. (2005) reported that β-glucan decreased the release of IL-1β because it inhibited the production of arachidonic acid metabolite. PGE_2 modulates many aspects of inflammation and immune response including inhibition of lymphocyte proliferation (Harris et al., 2002), inhibition of IL-2 synthesis by T cells (Katamura et al., 1995), and bi-directional modulation of T cell-dependent antibody production (He et al., 2002). Therefore, we speculate that β-1,3/1,6-glucan increased lymphocyte proliferation and serum IgG concentration possibly by inhibiting PGE_2 synthesis.

Ghrelin, a novel growth-hormone-releasing acylated peptide, was isolated from the mammalian stomach (Kojima et al., 1999; Hayashida et al., 2001). Recent works suggest that ghrelin plays an important role in hormone secretion, energy homeostasis, body weight control, and food intake in mammals (Nakazato et al., 2001). Salfen et al. (2004) reported that ghrelin might positively influence weight gain and concomitantly increase GH, insulin, and cortisol secretion in weaned pigs. The study was designed to examine the effects of β-1,3/1,6-glucan on plasma ghrelin and GH levels, and to explore whether β-1,3/1,6-glucan influences the somatotropic axis of weanling piglets. In this study, β-1,3/1,6-glucan was found to have no effect on GH concentrations, which agreed with the reports of Mao et al. (2005). However, we also observed that weanling piglets fed 50 mg/kg of β-1,3/1,6-glucan had a numerical increase in ghrelin concentration on d 28, and a similar change trend with ADG from d 14 to 28. The exact relationship between β-1,3/1,6-glucan and ghrelin needs further investigation.

CONCLUSION

In conclusion, ADG and feed efficiency had a quadratic increase trend with dietary β-1,3/1,6-glucan supplementation from d 14 to 28, and had no significant on ADFI. The treatment group fed 50 mg/kg dietary β-1,3/1,6-glucan supplementation had a numerical increase in ghrelin, and a similar change trend with ADG, whereas no significant effect on GH was observed. Lymphocyte proliferation indices, serum IgG and plasma PGE_2 concentrations varied linearly with dietary supplementation levels of β-1,3/1,6-glucan on d 14. Higher levels of β-1,3/1,6-glucan may have a transient immunoenhancing effect on the cellular and humoral immune function of weanling piglets via decreased PGE_2. Taking into account

immune response and growth performance, the most suitable dietary supplementation level of β-1,3/1,6-glucan is 50 mg/kg for weanling piglets. However, further research is necessary to investigate the relationship between β-1,3/1,6-glucan and ghrelin.

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