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Short communication

Effects of dietary supplementation of chitosan on humoral and cellular immune function in weaned piglets

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ABSTRACT

The effects of dietary supplementation of chitosan on humoral and cellular immune function in weaned piglets were investigated. One hundred and eighty piglets weaned at 28 d (Duroc × Large white × Landrace) were assigned randomly to 5 dietary treatments with 6 repetitions in each treatment. The piglets in the 5 treatments were fed on the basal diet supplemented with 0 (control), 100, 500, 1000 and 2000 mg chitosan/kg feed. Results showed that chitosan improved serum immunoglobulin G (IgG) concentrations of piglets in a quadratic dose-dependent manner ($P<0.05$), and increased serum specific ovalbumin (OVA) IgG contents in a linear or a quadratic dose-dependent manner ($P<0.05$) on day 28, while serum immunoglobulin A (IgA) and immunoglobulin M (IgM) concentration were not altered. With increasing chitosan, the secretory immunoglobulin A (sIgA) was enhanced in ileum mucosal surfaces in a linear or quadratic manner ($P<0.05$) on day 14, and was improved quadratically in jejunum mucosal surfaces on day 28 ($P<0.05$). In addition, chitosan decreased serum concentrations of soluble CD4 (sCD4) in a quadratic dose-dependent manner ($P<0.05$) and soluble CD8 (sCD8) in a linear or quadratic dose-dependent manner ($P<0.05$) on day 28. Chitosan quadratically enhanced serum concentrations of interleukin-1 (IL-1) and interleukin-2 (IL-2) on day 14 as well as serum concentrations of tumor necrosis factor-alpha (TNF-α) on day 28 ($P<0.05$). These results implied that dietary supplement with chitosan improved humoral and cellular immune responses of weaned piglets in a dose-dependent manner, and in this experiment, the appropriate adding dose of chitosan might be between 500 and 1000 mg/kg.

1. Introduction

The immune system of piglet is underdeveloped fully at early age (Heugten et al., 1996), and the ability to resist disease mainly depends on passive immunity from the sow during this time (Rooke and Bland, 2002). Early weaning not only interrupts the supply of immunologically important factors from sow's milk (Wu et al., 2004), but also impairs the production of antibodies and compromises cellular immune functions (Touchette et al., 2002), which leads piglets to be infected more easily by pathogens. Traditionally, antibiotics were frequently used in the diets of newly weaned pigs for the prophylaxis of infections during the immediate post-weaning period in past decades (Bosi et al., 2011). However, there has been increasing

Abbreviations: IgG, immunoglobulin G; IgM, immunoglobulin M; IgA, immunoglobulin A; sIgA, secretory immunoglobulin A; sCD4, soluble CD4; sCD8, soluble CD8; IL-1, interleukin-1; IL-2, interleukin-2; TNF-α, tumor necrosis factor-alpha.

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Table 1
Composition and nutrient levels of the basal diet (air dry basis, %).

Ingredients	Content	Nutrients	Level
Corn	51.90	Digestible energy (MJ/kg)	14.32
Soybean meal	16.00	Crude protein	20.02
Wheat	20.00	Crude fat	3.0
Fish meal	2.50	Crude fibre	4.2
Corn gluten meal	2.00	Calcium	0.72
Whey powder	2.00	Phosphorus	0.56
Soya bean oil	2.00	Lysine	1.35
Limestone	0.70	Methionine + Cystine	0.82
$CaHPO_4$	1.00	Threonine	0.74
NaCl	0.30		
Premix[a]	1.60		
Total	100		

[a] The premix provides following nutrients per kg diet: Vitamin A, 16,000 IU; Vitamin D3, 2500 IU; Vitamin E, 60 IU; Vitamin K3, 4.5 mg; Vitamin B1, 2.6 mg; Vitamin B2, 8.7 mg; Vitamin B6, 7.0 mg; Vitamin B12, 0.03 mg; vitamin C, 200 mg; Pantothenic acid, 13 mg; Nicotinic acid, 35 mg; Biotin, 0.47 mg; Folic acid, 0.85 mg; Iron, 155 mg; Copper, 35 mg; Zinc, 100 mg; Manganese, 25 mg; Iodine, 0.35 mg; Cobalt, 0.2 mg; Selenium, 0.25 mg; Choline chloride, 750 mg; Phytase, 500 FTU.

pressure on the livestock industry to decrease or discontinue these additions because of the potential development of antibiotic resistance (Davis et al., 2004). Therefore, alternative additives that help develop the immune responses of weaned piglets are highly recommended.

Chitosan, a natural and nontoxic alkaline polysaccharide, is formed by the action of chitin deacetylases and is a key structural component of helminths, arthropods and fungi (Synowiecki and Al-Khateeb, 2003). Porporatto et al. (2005) demonstrated that chitosan profoundly affected intestinal mucosal immunity by activating leukocytes. Our previous study found that chitosan improved the humoral and cellular immune functions in broilers (Li, 2009).

In piglets, however, there were limited studies evaluating the effect of chitosan on immune function. Therefore, our study was conducted to determine the effect of chitosan on the humoral and cellular immune function of weaned piglets and the appropriate chitosan supplemental level as an immuno-modulating agent.

2. Materials and methods

All procedures described in this experiment were approved by Animal Care and Use Committee of Inner Mongolia Agricultural University.

2.1. Experimental design and animal management

A total of 180 piglets (Duroc × Large white × Landrace) with an initial average body weight of 7.6 kg were assigned randomly to 5 treatments with 6 repetitions (3 pens of males and 3 pens of females) in each treatment, with 6 piglets in each pen (4.0 m × 4.2 m). The formation of basal diets was showed in Table 1. All diets were offered in meal form. Five dietary treatments supplemented with 0 (control), 100, 500, 1000 or 2000 mg chitosan/kg feed on the basal diet, respectively. Piglets were weaned at the age of 28 d, penned in a temperature-controlled nursery building where temperature was maintained at 26–27 °C and relative humidity was about 65–70%. The weaned piglets had one week of housing and management adaptation before the experimental phase. The experimental period was 28 d. Feed and water were freely available. Chitosan used in this trial was provided by Jinan Haidebei Marine Bioengineering Limited Company (Jinan, China). The deacetylation degree of chitosan was determined to be 85.09%, and the viscosity was 45 cps.

2.2. Sample collection

On day 14 and 28, one pig from each replicate of each treatment was randomly selected and blood samples were obtained by puncturing the vena cava. The blood samples were centrifuged at 3000 × *g* for 20 min at 4 °C to yield serum. Serum was stored at −20 °C until analysis of immunoglobulins, cytokines and sCD4, sCD8. At the beginning of trial, one piglet from each repetition of each treatment was selected randomly and injected with 1 mg ovalbumin/kg BW (Sigma, USA). Blood samples were collected by puncturing the vena cava on day 0 (before injection), 14 and 28 to test the specific OVA antibody concentrations in serum. The blood samples were centrifuged at 3000 × *g* for 20 min and stored at −20 °C until analysis. For determining the content of sIgA in small intestine mucosa, the pigs used to get blood samples were sacrificed, and the jejunum and ileum were quickly removed, and then the samples were cut and washed with PBS (pH 7.2–7.4). Intestinal mucosa was gently scraped with slide, weighed 1 g and transferred to a centrifuge tube adding 9 mL saline then homogenized by hand. Homogenates were centrifuged at 3000 × *g* for 20 min, and the supernatant was stored at −20 °C until analysis.