

$\beta(1-3)(1-6)$ -D-glucans modulate immune status in pigs: potential importance for efficiency of commercial farming

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Background: In face of the challenge of the emergent diseases and the current efforts of the governments to create conditions to ban growth-promoting antibiotics and to improve efficiency of the commercial farming, new opportunities are created for natural, highly effective and cost affordable immunomodulators; able to induce and enhance resistance against diseases and to reduce farming-related stress. Supplementation of animal feed with $\beta(1-3)(1-6)$ -D-glucans has been repeatedly shown to modulate the immune system and to influence growth characteristics of farmed animals.

Materials and methods: In our study we focused on evaluation of effects of an insoluble, fungi-derived $\beta(1-3)(1-6)$ -D-glucan as dietary supplement in piglets. We measured the growth, phagocytosis of peripheral blood cells and interleukin 2 (IL-2) and tumor necrosis factor alpha (TNF- α) production after feeding with 15 mg of glucan/kg/day.

Conclusions: Following supplementation, $\beta(1-3)(1-6)$ -D-glucan has been shown to stimulate growth, phagocytic activity, and IL-2 production. In addition, it significantly lowered the cortisol and TNF- α levels after lipopolysaccharide (LPS) challenge.

Keywords: β -glucan; fungi; immunity; pigs; phagocytosis; tumor necrosis factor alpha (TNF- α)



Submitted Nov 20, 2013. Accepted for publication Jan 20, 2014.

doi: 10.3978/j.issn.2305-5839.2014.01.04

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Introduction

Immune system is a system of the biological structures, pathways and mechanisms of mutually interactive systems of an organism that help to protect it against diseases and infections. Initially, $\beta(1-3)(1-6)$ -D-glucans (term β -glucan will be used throughout the paper) have drawn so much attention worldwide due to their immunomodulatory capabilities in the innate immune system. Numerous types of β -glucans have been isolated from fungi (yeasts, molds and mushrooms), grain and seaweed. They have been extensively studied for their immunological and pharmacological effects and currently more than 4,000 papers describing the mostly immunological activities of β -glucans exist in the literature (1).

The innate immune system is fairly well conserved among vertebrates due to its ancient roots in evolutionary history. It

is estimated that the innate system goes as far back as early metazoan—over a billion years ago (2,3). This retention through time of the innate system explains the effects of β -glucans throughout the animal species. Although β -glucan was initially considered to be stimulator of the cellular immunity, in the last decades researchers demonstrated that its biological effects are pleiotropic. On one hand, they are able to absorb a wide range of mycotoxins (4), on the other hand, they stimulate both branches of immunity. In addition, β -glucans were shown to reduce serum cholesterol in hypercholesterolemic animals (5), suppress cancer growth (6), lower stress (7), and regulate blood sugar levels (8). β -Glucan has routinely been used in animal farming; in fish (9), shrimp (10) and in pig feed (11-13). The addition of β -glucan to pig feed altered immune and intestinal functions (12), increased weight (14), reduced

Table 1 Diet composition

Diet composition	Percent (%)
Protein	16.00
Fat	4.00
Crude fiber	4.10
Lysine	0.77
Methionine	0.28
Calcium	0.80
Phosphorus	0.30
Chloride	0.40
Sodium	0.23
Alanine	0.90
Arginine	0.96
Cystine	0.24
Glycine	0.92
Histidine	0.41
Isoleucin	0.68
Leucin	1.30
Phenylalanine	0.72
Tyrosine	0.45
Threonine	0.57
Tryptophan	0.19
Valine	0.78
Serin	0.80
Aspartic acid	1.45
Glutamic acid	3.92
Potassium	0.75
Magnesium	0.19
Sulfur	0.20

Based on manufacturer's information. For all experiments, we used Laboratory Porcine Diet Grower #5084 (Purina Mills, Inc., Richmond, IN).

peak net glucose flux (15), decreased T_h17-related cytokine production (16) and protected against infection (17). In chicken, β -glucan addition was found to reduce the severity of *Eimeria* infection (18), to upregulate the phagocytosis of macrophages after bacterial infection (19) and to improve growth performance (20).

In this paper, we decided to evaluate the effects of adding β -glucan into commercial feed of piglets. We measured weight, changes in phagocytosis and levels of interleukin 2 (IL-2), cortisol and tumor necrosis factor alpha (TNF- α) in blood. Phagocytosis and IL-2 levels are two of the most often

used immunological responses tested in glucan studies, LPS challenge is particularly important in farmed animals.

The objective of this study was to evaluate the effects of this β -glucan on biological characteristics important for health of these farm animals.

Materials and methods

Animals

At the onset of the study, the animals were examined for signs of any disease and all were considered healthy on the basis of a lack of clinically relevant abnormalities. The animals were then randomly assigned to individual groups. None of the used animals developed any disease including lethargy, fever or gastrointestinal problems during this study. Similarly, none of these animals either died or were euthanized during this study. The use of animals was approved by the University of Louisville Institutional Animal Care And Use Committee (IACUC) (#12029). Piglets (all white Yorkshire-Landrace) were purchased from Oak Hill Genetics (Ewing, IL, USA). All animals were grown in conventional conditions.

Materials

Lipopolysaccharides (LPS) from *Escherichia coli*, and sodium citrate were obtained from Sigma (St. Louis, MO, USA).

Glucan

Insoluble β -glucan is 68.5% pure and isolated from *Sacharomyces cerevisiae* (Biorigin, Brazil).

Feed

For all experiments, we used Laboratory Porcine Diet Grower #5084 (Purina Mills, Inc., Richmond, IN). The composition of the diet is given in *Table 1*. After weaning, piglets were placed in individual pens. Weekly body weight was recorded and treatment dosages adjusted accordingly. Piglet feed was supplemented with 15 mg of β -glucan/kg/day.

Phagocytosis

The technique employing phagocytosis of synthetic polymeric microspheres was described earlier (21,22).

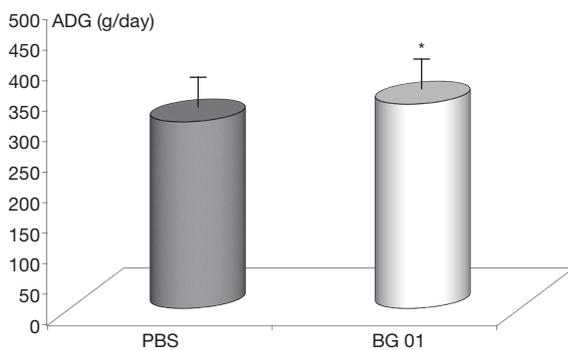


Figure 1 Average daily gain. At least five pigs were evaluated at each experimental group. Each value represents the mean \pm standard deviation (SD). *, represents significant differences from control phosphate buffered saline (PBS), values at $P \leq 0.05$ level.

Briefly: 0.1 mL of peripheral blood (with sodium citrate 1:9) or 0.1 mL of peritoneal fluid was incubated *in vitro* with 0.05 mL of 2-hydroxyethyl methacrylate particles (HEMA particles; 5×10^8 /mL). The test tubes were incubated at 37 °C for 60 min, with intermittent shaking. Smears were stained with Wright stain. The cells with three or more HEMA particles were considered positive. All experiments were performed in triplicates. At least 300 cells in 60 high power fields were examined in each experiment.

IL-2

Levels of IL-2 were evaluated in serum (1 mL of blood was collected) at the end of experiment using a commercial IL-2 enzyme-linked immunosorbent assay (ELISA) kit as recommended by the manufacturer (R&D Systems, Minneapolis, MN, USA) for pigs.

Evaluation of TNF- α

Levels of TNF- α were evaluated in serum (1 mL of blood was collected) at 1, 2 and 3 hr. after injection of LPS from 14 days after beginning of feedings with β -glucan. The level of TNF- α , Rockford, IL, USA).

Evaluation of cortisol

Levels of cortisol were evaluated in serum (1 mL of blood was collected) at 1, 2 and 3 hr. after injection of LPS from 14 days after beginning of feedings with β -glucan. The levels of cortisol were measured by a commercial ELISA kit (Abnova, Walnut, MA, USA).

Weight

Piglets were fed with the diet (Table 1) with or without β -glucan for 28 days. Weights were determined weekly to calculate average daily gain (ADG) and average daily feed intake (ADFI); data not shown.

Statistics

Data were analyzed by Analysis of Variance (ANOVA) using the General liner model (GLM) procedure appropriate for a factorial arrangement of treatments in a randomized block design. The statistical model included the effects of challenge (LPS) and/or dietary supplements. The pen was used as the experimental unit for analysis. A level of $P \leq 0.05$ was used to indicate statistically significant results.

Results

Body weights were recorded every week for the entire duration of the experiment. ADGs were calculated for each of the 7-day periods. The ADG was influenced by the addition of β -glucan. Addition of glucan caused significant increase in piglet model (Figure 1). Average daily food intake did not increase (data not shown).

β -Glucans are considered non-specific stimulators of cellular immunity, particularly macrophages. Phagocytosis is therefore one of the main defense reactions, where a stimulating effects of β -glucan can be observed. For our experiments, we used phagocytosis of synthetic microspheres based on 2-hydroxyethyl methacrylate with minimal false positivity. Our results showed that addition of β -glucan significantly potentiated phagocytic activity of both peripheral blood monocytes and neutrophils (Figure 2). Experiments evaluating the effects of glucan on IL-2 production in blood showed that feeding with β -glucan significantly increased the level of IL-2 (Figure 3).

The next two experiments measured the effects of β -glucan supplementation of the body response after a LPS challenge. First, we tested the levels of the stress hormone cortisol. In piglets, glucan significantly abolished the effects of stress (Figure 4). Basal levels of cortisol were 112 nmol/L.

In the last part of our study, we measured concentrations of TNF- α our experimental model, addition of the β -glucan to the feed resulted in significant reduction of TNF- α levels in all three tested intervals (Figure 5). Basal levels of TNF- α

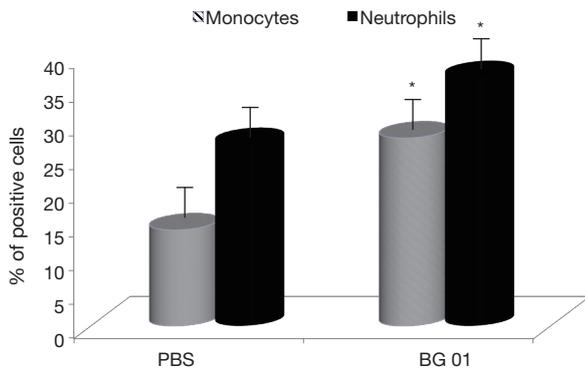


Figure 2 Effect of dietary supplementation with β -glucan samples on phagocytosis by peripheral blood monocytes and neutrophils of pigs. Each value represents the mean \pm standard deviation (SD). *, represents significant differences between control phosphate buffered saline (PBS) and tested samples at $P \leq 0.05$ level.

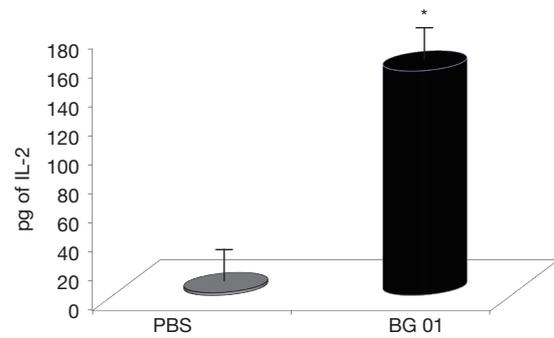


Figure 3 Effect of dietary supplementation with β -glucan samples on interleukin 2 (IL-2) level in serum of pigs. Each value represents the mean \pm standard deviation (SD). *, represents significant differences between control phosphate buffered saline (PBS) and tested samples at $P \leq 0.05$ level. All experiments were performed in triplicates.

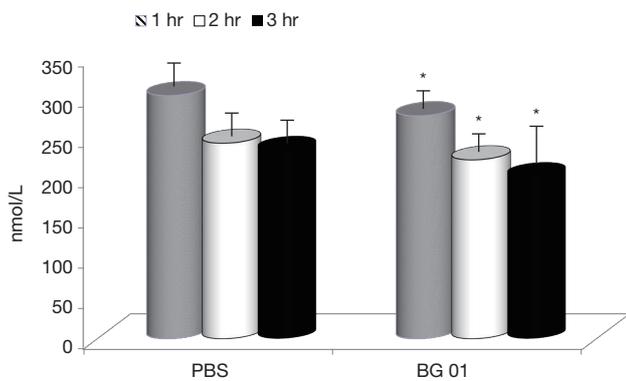


Figure 4 Effect of addition of β -glucan to feed on lipopolysaccharides (LPS) challenge-induced levels of corticosterone in pigs. *, represents significant differences between stressed-control phosphate buffered saline (PBS) and glucan samples at $P \leq 0.05$ level.

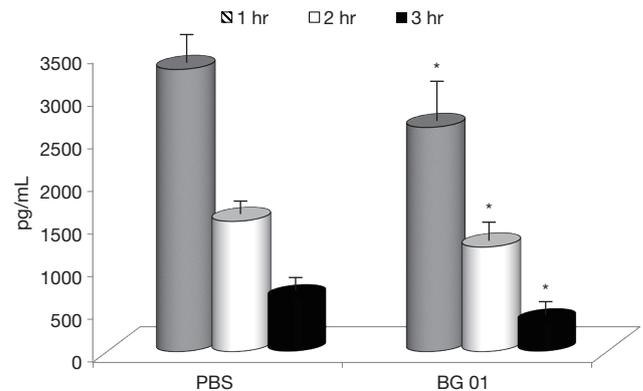


Figure 5 Effect of addition of β -glucan to feed on lipopolysaccharide challenge-induced levels of tumor necrosis factor alpha (TNF- α) in pigs. *, represents significant differences between stressed-control phosphate buffered saline (PBS) and glucan samples at $P \leq 0.05$ level.

were zero.

Discussion

The present study evaluates the effects of insoluble β -glucan on the various reactions in pigs. We found higher ADG when given β -glucan, which is in agreement with previous findings (23-25). The exact mechanisms for these effects are not clear, once the energetic contribution of the β -glucans to the feed at dosages used is neglectable. Plausible explanations might be stress reduction, effects on gastrointestinal tract microbiota (26), on gut permeability (12) and reduction of the negative effects of LPS on feed intake.

With respect of immune system of farm animals, it must be considered that these animals are naturally challenged by LPS during their production period. Even in simpler events, as subclinical diseases or high fiber feeding, higher levels of LPS are produced. LPS will stimulates proinflammatory response, higher TNF-induced cell monolayer disruption and higher tight junction permeability to the intestinal epithelium, leading to higher leakage of LPS to the blood and the initiation of a vicious cycle of proinflammatory response (27). It has a potential to impair feed intake and immune status resulting in the reduction of the production efficiency (28).

β -glucan is well known to be a significant stimulator of

immune reaction, particularly of the cellular branch. These effects were repeatedly observed regardless the route of administration (29). For evaluation of the potential effects of our glucan on phagocytosis, we used synthetic polymer microparticles based on hydroxymethacrylate, known for their low negative charge and therefore minimal subjective error during evaluation (22). Our results showed significant stimulation of phagocytosis by both peripheral blood monocytes and neutrophils, which is in agreement with previous finding in mice (29).

The second part of our study focused on effects of β -glucan after an endotoxin challenge. We found significantly lower levels of both cortisol and TNF- α . These results are in agreement with data found in similar model of LPS challenge after weaning (23). The glucan effects found in our study were even stronger than those reported by (24), which might be caused by use of different β -glucans.

In conclusion, β -glucans had significant immunomodulating effects in piglet, strongly suggesting that supplementation of feed with this glucan results in improvement of biological and immunological conditions of farmed animals. In addition, with current efforts to ban on antibiotic and growth promoters, the use of β -glucans as feed additive offers two benefits—natural growth stimulation and natural protection (13).

Acknowledgements

Disclosure: The authors declare no conflict of interest.

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Cite this article as: Vetvicka V, Oliveira C. β (1-3) (1-6)-D-glucans modulate immune status in pigs: potential importance for efficiency of commercial farming. *Ann Transl Med* 2014;2(2):16. doi: 10.3978/j.issn.2305-5839.2014.01.04