

## Effects of $\beta$ -Glucan Supplementation on Lymphocyte Proliferation, Macrophage Chemotaxis and Specific Immune Responses in Broilers

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**ABSTRACT :** Immunomodulatory feed additives might offer alternatives to antimicrobial growth promoters in poultry production. This experiment was carried out to test the effect of  $\beta$ -glucan supplementation on the growth performance and immune response in broilers. Total of 160 day-old broilers were randomly assigned to 4 treatment groups fed corn-soybean diets containing 0, 0.012, 0.025 or 0.05% of  $\beta$ -glucan supplement in a 6 week feeding experiment. Growth performance, antibody titer against New Castle vaccine, lymphocyte blastogenesis, and peritoneal macrophage chemotaxis activity of broilers were evaluated. Results showed that there were no significant differences in weight gain and feed efficiency among the treatments, and no differences in antibody titer was observed. Supplementation of  $\beta$ -glucan did not elevate the lymphocyte blastogenesis among treatments, following stimulation with different mitogens. However, supplementation with 0.025 and 0.05%  $\beta$ -glucan enhanced the macrophage chemotaxis activity of broilers. These results suggest that  $\beta$ -glucan may enhance some cell-mediated immune responses of chickens by modulate macrophages ability. (*Asian-Aust. J. Anim. Sci.* 2004, Vol 17, No. 8 : 1145-1149)

**Key Words :**  $\beta$ -glucan, Lymphocyte proliferation, Macrophage chemotaxis, Immune responses, Broilers

### INTRODUCTION

(1,3)- $\beta$ -D-glucans (glucans) is a heterogenous group of glucose polymers present as structural elements in the cell walls of yeast, fungi and cereals. Biological activities of these molecules have been reported to inhibit tumor formation (Ohno et al., 1995), enhance defense against bacterial challenge (Onderdonk et al., 1992; Babineau et al., 1994), and increase growth performance (Schoenherr et al., 1994) in pigs. Glucans from a variety of yeast cell walls have been shown to stimulate both specific and non-specific immune responses (salmon, Robertsen et al., 1990; Pigs, Williams et al., 1994). *In vitro* studies have shown that these molecules influence macrophage morphology (Burgaleta et al., 1978), increase nitric oxide production (Ohno et al., 1996), and release some kind of cytokines, such as TNF- $\alpha$ , IL-6, IL-1 and IL-2 (Adachi et al., 1994; Chen et al., 2003). Glucans could also decrease inflammatory cytokine responses (Poutsika et al., 1993), thereby allowing nutrients to be partitioned toward growth demand (Klasing et al., 1987).

Due to the ban of most antibiotics growth promoters in the Europe and the expected spread of this trend to the rest of the world, finding alternative means of boosting disease resistance without the antibiotics is considerable practical

interest of animal production. In this context, the use of immune modulators for increasing host defense and immunocompetence is receiving more attention.

Although glucans have not been widely used in veterinary practice and animal production, Liu and Li (1999) reported that lentinan (a type of glucan) has effects on enhancing interleukin-2 (IL-2) production of broilers infected by Marek's virus. It was suggested that lentinan may be useful in treating against Marek's disease and other viral diseases because of its potential antitumor and immunostimulating activities. However, little is known about the effects of  $\beta$ -glucans on the immunity of broilers. The aim of the present study was to investigate the effects of dietary  $\beta$ -glucans on the growth performance, lymphocytes blastogenesis and macrophage functions in broilers.

### MATERIALS AND METHODS

#### Animal and diets treatment

A total of 160 day-old broilers (half male and half female) were obtained from a commercial hatchery. They were randomly allotted into four dietary treatments containing 0, 0.012, 0.025 and 0.05%  $\beta$ -glucan. Then birds were raised in electrically heated brooders with 10 chicks per pen, 2 pens for each treatment, and repeat once. The basal diet (Table 1) was base on corn and soybean meal and was formulated according to NRC (National Research Council, 1994). The  $\beta$ -1,3-glucan was derived from *Schizophyllum commune* and was obtained from Taito Co. (Tokyo, Japan). Feed and water were provided *ad libitum* throughout the 6 wk experimental period. Chickens were

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**Table 1.** The composition and calculated analysis of basal diet (%)

| Ingredients                               | Growers<br>(0-3 wks) | Finishers<br>(4-6 wks) |
|---|----------------------|------------------------|
| Corn                                      | 46.00                | 56.90                  |
| Soybean meal                              | 43.41                | 34.13                  |
| Soybean oil                               | 6.94                 | 5.30                   |
| Dicalcium phosphate                       | 1.58                 | 1.40                   |
| Limestone                                 | 1.35                 | 1.50                   |
| Salt                                      | 0.40                 | 0.40                   |
| DL-methionine                             | 0.15                 | 0.07                   |
| Choline chloride                          | 0.02                 | 0.05                   |
| Vitamin premix                            | 0.10                 | 0.10                   |
| Mineral premix                            | 0.10                 | 0.10                   |
| Coccidiostat                              | 0.05                 | 0.05                   |
| Total                                     | 100.00               | 100.00                 |
| Calculated nutrient composition           |                      |                        |
| Crude protein                             | 23                   | 20                     |
| Metabolizable energy, kcal/kg             | 3,200                | 3,200                  |
| sulfur-containing amino acid<br>(Met+cys) | 0.84                 | 0.71                   |
| Lysine                                    | 1.30                 | 1.10                   |
| Calcium                                   | 1.00                 | 1.00                   |
| Available phosphorus                      | 0.45                 | 0.40                   |

<sup>a</sup> Supplied per kg diet: vitamin A, 8,000 IU; vitamin D<sub>3</sub>, 1,200 ICU; vitamin E, 10 IU; vitamin K, 2 mg; thiamin, 2 mg; riboflavin, 5 mg; pyridoxine, 0.2 mg; vitamin B<sub>12</sub>, 0.03 mg; Ca-pantothenate, 10 mg; niacin, 50 mg; biotin, 0.1 mg; folic acid, 0.5 mg; choline, 300 mg; Mn, 50 mg; Zn, 60 mg; Cu, 10 mg; Se, 0.15 mg; Fe, 80 mg.

bled from cardiac puncture at 3 and 6 wks of age. The blood was for subsequently analyzed antibody titer and lymphocyte blastogenesis. Body weights, feed intake and feed conversion by pen were recorded at weekly intervals. Mortality was recorded when occurred. The experimental protocol was approved by National I-Lan University Experimental Animal Committee.

#### Lymphocytes blastogenesis assays

Whole blood Lymphocytes proliferation assay was described as Talebi et al. (1995). Briefly, blood with anticoagulant were drained, and the separated lymphocytes ( $1 \times 10^7$  cells/0.2 ml/well) were cultured in triplicates with RPMI-1640 growth medium supplemented with 10% heat inactivated fetal bovine albumin and antibiotics (penicillin, 100 U/ml and streptomycin, 50 µg/ml), in a 96 well plate in a 39°C humidified chamber with 5% CO<sub>2</sub> and 95% air for 72 h. To determine mitogen stimulation of lymphocytes, different mitogens were used including 20 µg/ml lipopolysaccharides (LPS, 055:B5, Sigma, St. Louis, Mo), 35 µg/ml concanavalin A (Con A), and 24 µg/ml pokeweed mitogen (PWM). The concentrations of used mitogens have been test and shown optimal proliferation in our preliminary study (Cheng and Pang, 1996). Lymphocytes were pulsed with 0.2 µCi/well <sup>3</sup>H-thymidine (Specific activity 80 Ci/ml, Amstersham Pharmacia Biotech,

Sweden) for another 18 h incubation, and the cells were harvested onto a glass filter. <sup>3</sup>H-thymidine incorporation was determined by using luminescence counter (Packard, USA) and data are presented as cpm value basis, due to the previous study showed that <sup>3</sup>H-thymidine incorporation into lymphocyte was not significantly difference from the dietary treatments. Three birds from each treatment were used for the blastogenesis assay.

#### Harvest of macrophages

Sephadex-elicited peritoneal exudative cells (PECs), the source of macrophages, were obtained by the method described by Trembicki et al. (1984). Briefly, a 3% swollen Sephadex-G50 suspension in sterile saline (0.75%) was injected intraperitoneally into each bird at 1 ml per 100 g body wt. Approximately 42 h after injection, the birds were euthanasia, and PECs were collected by flushing the peritoneal cavity with 30 ml of sterile heparin (0.5 U/ml) saline solution. The peritoneal exudates were centrifuged at 285×g at 4°C for 15 min. The resulting PEC pellets were resuspended in 2 ml complete medium consisting of RPMI-1640 growth medium supplemented with 10% heat inactivated fetal bovine serum and antibiotics (penicillin, 100 U/ml and streptomycin, 50 µg/ml). The viable numbers of PECs were determined by trypan blue exclusion on a hemocytometer, and the cell density was adjusted to  $2 \times 10^6$  PECs/ml. Six birds from each treatment were used for the harvest of macrophages.

#### Nitric oxide production of peritoneal macrophages

The macrophages from different treatments were stimulation with 1 mg/ml of LPS for 0, 3 and 6 h respectively. The accumulation of NO in culture supernatants was measured by following the Ding et al. (1988) described method. Briefly, 100 µl of supernatant was pipetted from each well into an empty 96 well plate. After the addition of 100 µl Griess reagent to each well, absorbance at 540 nm was measured by using a ELISA reader (Tecan, Maennedorf, Switzerland). NO concentration was calculated from a NaNO<sub>2</sub> standard curve. Griess reagent was prepared by mixing one part of 0.1% naphthylethylene diamine dihydrochloride in distilled water plus one part of 1% sulfanilamide in 5% concentrated H<sub>3</sub>PO<sub>4</sub>.

#### Measurements of antibody titers

Antibody titers of Newcastle disease (ND) were determined by ELISA reader using the Idexx test kit (IDEXX, Maine, USA). For blood biochemical assay, blood from 3 or 6 week-old chicks per treatment (8 birds/treatment) were collected by cardiac puncture, and the blood were centrifuged at 2,654×g for 10 min.

**Table 2.** Effects of  $\beta$ -glucan supplementation on the growth performance of broilers

|                               | 0%              | $\beta$ -glucan |                 |                  |
|-------------------------------|-----------------|-----------------|-----------------|------------------|
|                               |                 | 0.012%          | 0.025%          | 0.05%            |
| 0-3 week                      |                 |                 |                 |                  |
| Feed intake <sup>1</sup>      | 1.78 $\pm$ 1.20 | 1.95 $\pm$ 1.35 | 1.85 $\pm$ 1.33 | 1.81 $\pm$ 1.25  |
| Body weight gain <sup>1</sup> | 0.61 $\pm$ 0.04 | 0.67 $\pm$ 0.03 | 0.66 $\pm$ 0.03 | 0.62 $\pm$ 0.004 |
| Feed efficiency <sup>2</sup>  | 0.46 $\pm$ 0.33 | 0.44 $\pm$ 0.29 | 0.48 $\pm$ 0.36 | 0.45 $\pm$ 0.31  |
| 4-6 week                      |                 |                 |                 |                  |
| Feed intake <sup>1</sup>      | 1.81 $\pm$ 1.31 | 1.86 $\pm$ 1.32 | 1.91 $\pm$ 1.30 | 1.72 $\pm$ 1.19  |
| Body weight gain <sup>1</sup> | 1.60 $\pm$ 0.36 | 1.67 $\pm$ 0.53 | 1.78 $\pm$ 0.46 | 1.75 $\pm$ 0.40  |
| Feed efficiency <sup>2</sup>  | 1.09 $\pm$ 0.59 | 1.06 $\pm$ 0.47 | 1.11 $\pm$ 0.52 | 1.23 $\pm$ 0.62  |

<sup>1</sup>(kg/bird). <sup>2</sup>weight gain/kg feed. Data presented as mean $\pm$ SD (n=10). None of the differences among treatments were statistically significant (p>0.05).

**Table 3.** Effects of  $\beta$ -glucan on supplementation lymphocytes blastogenesis in broilers

| Mitogen  | Treatment                          |                                     |                                     |                                |
|----------|------------------------------------|-------------------------------------|-------------------------------------|--------------------------------|
|          | 0%                                 | $\beta$ -glucan                     |                                     |                                |
|          |                                    | 0.012%                              | 0.025%                              | 0.05%                          |
| 0-3 week |                                    |                                     |                                     |                                |
|          |                                    | cpm (count per minute)              |                                     |                                |
| LPS      | 37.3 $\pm$ 28.7                    | 35.8 $\pm$ 22.9                     | 29.5 $\pm$ 10.3                     | 17.5 $\pm$ 3.4                 |
| Con A    | 605.6 $\pm$ 410.8                  | 261.8 $\pm$ 315.7                   | 572.3 $\pm$ 975.9                   | 72.8 $\pm$ 93.8                |
| PWM      | 48.0 $\pm$ 17.5                    | 76.3 $\pm$ 63.7                     | 45.3 $\pm$ 29.6                     | 36.6 $\pm$ 29.2                |
| 4-6 week |                                    |                                     |                                     |                                |
|          |                                    | cpm (count per minute)              |                                     |                                |
| LPS      | 230.8 $\pm$ 138.7 <sup>a</sup>     | 85 $\pm$ 48.4 <sup>b</sup>          | 131.5 $\pm$ 79.4 <sup>ab</sup>      | 119.8 $\pm$ 84.2 <sup>ab</sup> |
| Con A    | 3,972.5 $\pm$ 2,060.6              | 2,415.8 $\pm$ 2,394.7               | 3,386.5 $\pm$ 2,180.9               | 1825.8 $\pm$ 2492.1            |
| PWM      | 2,959.3 $\pm$ 1,927.4 <sup>a</sup> | 2,142.8 $\pm$ 1,888.4 <sup>ab</sup> | 2,379.5 $\pm$ 1,515.9 <sup>ab</sup> | 724.1 $\pm$ 820.2 <sup>b</sup> |

Data presented as mean $\pm$ SD (n=6). Lipopolysaccharide (LPS), 20  $\mu$ g/ml; concanavalin A (Con A), 35  $\mu$ g/ml; pokeweed mitogen (PWM), 24  $\mu$ g/ml.

<sup>a, b</sup> Means in the same row without common superscripts differ significantly (p<0.05).

### Statistical analysis

The data were analyzed statistically according to the General Linear Model procedure of SAS (SAS, 1989). The percentage data were subjected to arc sin transformation prior to analysis of variance and differences among were separated by using Duncan's multiple range test.

## RESULTS AND DISCUSSION

### Growth performance

The effects of  $\beta$ -glucans supplementation on the growth performance of broilers are summarized in Table 2. No significant differences were observed among treatments in feed intake, weight gain and feed efficiency. These results imply that  $\beta$ -glucans had no effect on broiler performance even up to dosage of 0.05%. Similarly, Wu et al. (1997) observed no significant effects of  $\beta$ -glucans in daily gain feed intake in weanling pigs. But Dritz et al. (1995) found that  $\beta$ -glucans incorporated at 0.025% significantly improved the daily gain of weanling pigs than that of control group. Based on the nutrient distribution for growing chicken and immune response, nutrient flow will predominantly supply for antibody and cytokine synthesis (i.e. IL-1 and IL-2) when immune responses are primed. The final result will be growth depression (Klasing et al., 1987; Chen et al., 2003).

### Lymphocytes blastogenesis

Lymphocyte proliferation is an important indicator of lymphocyte function. In this study, different mitogens (LPS, Con A, and PWM) were applied to stimulate the proliferation of blood lymphocytes, therefore, mainly referring to the B, T, and B/T-lymphocyte proliferation, respectively. The influence of  $\beta$ -glucans on lymphocytes blastogenesis is summarized in Table 3. Data showed that LPS significantly (p<0.05) decreased the cpm value (85 $\pm$ 48.4) compared to the other two mitogens at 0.012%  $\beta$ -glucans level at 6 weeks of age. Significant decrease in cpm value (724.2 $\pm$ 820.2) also noticed after PWM challenge at 0.05%  $\beta$ -glucans level at 6 weeks of age. Overall our data suggest that  $\beta$ -glucans feeding for a 6 week period had no effect on lymphocytes proliferation following challenge with different mitogens, although, some dosages depressed the proliferation.

Recent reports show that lentinan (a type of  $\beta$ -glucans) could promote lymphocyte proliferation in broilers (Chen et al., 2003). However, lymphocyte proliferation indices were not found to be different between control and treatment groups (0.03%  $\beta$ -glucans) in weanling pigs in a 4 week feeding experiment (Hiss and Sauerwein, 2003). These inconsistent results may be due to the differences in the dosage and the type of  $\beta$ -glucans used. The mechanism by which  $\beta$ -glucans promotes lymphocyte proliferation is unclear and remains to be elucidated.

**Table 4.** Effects of  $\beta$ -glucan supplementation on the no. of harvested chickens peritoneal macrophages and nitric oxide production after exposure to lipopolysaccharides

| $\beta$ -glucan (%) | No. of macrophages<br>(No. $\times 10^7$ /bird) | Nitric oxide<br>( $\mu$ M)    |                               |                              |
|---------------------|---|-------------------------------|-------------------------------|------------------------------|
|                     |   | 3 h                           | 6 h                           | 12 h                         |
| 0                   | 0.53 $\pm$ 0.12 <sup>a</sup>                    | 6.30 $\pm$ 3.36 <sup>b</sup>  | 2.11 $\pm$ 0.91 <sup>bc</sup> | 1.46 $\pm$ 0.30 <sup>c</sup> |
| 0.012               | 0.56 $\pm$ 0.12 <sup>a</sup>                    | 9.13 $\pm$ 4.10 <sup>ab</sup> | 2.99 $\pm$ 0.70 <sup>b</sup>  | 2.41 $\pm$ 0.73 <sup>b</sup> |
| 0.025               | 0.57 $\pm$ 0.12 <sup>a</sup>                    | 11.48 $\pm$ 4.15 <sup>a</sup> | 4.50 $\pm$ 0.79 <sup>a</sup>  | 3.71 $\pm$ 0.67 <sup>a</sup> |
| 0.05                | 0.44 $\pm$ 0.12 <sup>a</sup>                    | 6.44 $\pm$ 3.50 <sup>b</sup>  | 1.86 $\pm$ 0.70 <sup>c</sup>  | 1.31 $\pm$ 0.45 <sup>c</sup> |

Data presented as mean $\pm$ SD (n=6). <sup>a, b, c</sup>Means in the same column without common superscripts differ significantly (p<0.05).

**Table 5.** Effects of  $\beta$ -glucan supplementation on antibody titers production after Newcastle disease challenge

|         | $\beta$ -glucan                 |                 |                 |                 |
|---------|---------------------------------|-----------------|-----------------|-----------------|
|         | 0%                              | 0.012%          | 0.025%          | 0.05%           |
|         | Log <sub>2</sub> titer $\pm$ SD |                 |                 |                 |
| 3 weeks | 2.95 $\pm$ 0.45                 | 3.11 $\pm$ 0.42 | 3.00 $\pm$ 0.48 | 3.19 $\pm$ 0.47 |
| 6 weeks | 2.84 $\pm$ 0.39                 | 3.15 $\pm$ 0.46 | 2.96 $\pm$ 0.52 | 3.26 $\pm$ 0.60 |

Data presented as means $\pm$ SD (n=6). Differences among treatments were not statistically significant (p>0.05).

### Chemotaxis and NO production of macrophages

Macrophage is a lineage of mononuclear phagocytic system and plays an important role in immune-defense against pathogenic agents (Skamene and Gros, 1983). Many functions are performed by macrophages, including phagocytosis of exogenous particles (Qureshi et al., 1986). Furthermore, macrophage also can secrete prostaglandins and cytokines to modulate the activity of lymphocytes and other macrophages (Kimball, 1990). Our results showed that the numbers of harvested peritoneal macrophages were not influenced by  $\beta$ -glucans supplementation (Table 4). The bacterial killing ability of macrophages was not evaluated in present study.

The NO concentration of macrophages reached plateau at the level of 0.025% glucan during 3-12 h *in vitro* culture, and then declined at 0.05% level (Table 4). Nitric oxide is a chemical messenger and has been recognized as an important effectors molecule for macrophages in their cytotoxic and cytostatic activity in fighting against invading pathogens and tumor cell targets (Liew, 1995). In addition, NO regulates host immunity as a modulator of T-lymphocyte response. The production of large amounts of NO by activated macrophages was found to suppress lectin-induced lymphocyte proliferation *in vitro* (Albina et al., 1991). There are some controversial reports about the immunomodulation of NO. Increased NO production is thought to be the result of increased gene expression of inducible NO synthase (iNOS) in macrophages by glucan stimulation, which is responsible for the synthesis of NO by activating NF- $\kappa$ B transcriptional factor as proposed by Ramamoorthy et al. (1996).

### Antibody titers

Specific vaccination responses, as quantified by the Newcastle disease (ND) antibody titers occurred in all birds,

but were not related to glucan feeding (Table 5). The results also indicated that antibody titers between 3 and 6 weeks of age were similar observed in Hiss and Sauerwein (2003) who challenged weanling pigs with PRRS (porcine reproductive and respiratory syndrome) vaccine after 0.03% glucan feeding for 4 weeks, and no benefit outcome was noted with glucan supplementation. But rats fed with glucan and challenge the parasite trophozoites via the intraperitoneal route increased serum IgG antibody titers by 300-fold (Navarro-Garcia et al., 2000). This dramatic induction of serum IgG antibody by IP co-administration of glucan and antigens may be due to macrophage activation. Since, our antigen challenge was by way of subcutaneous injection and glucan was provided by oral route, glucan may not have expressed its immune adjuvant characteristics.

Concluded our findings, showed that  $\beta$ -glucan up to 0.05% did not have significant differences in weight gain and feed efficiency among the treatments, and no differences in antibody titer was observed. Supplementation of  $\beta$ -glucan did not elevate the lymphocyte blastogenesis among treatments, following stimulation with different mitogens. However, supplementation with 0.025 and 0.05%  $\beta$ -glucan enhanced the macrophage chemotaxis activity of broilers. These results suggest that  $\beta$ -glucan may enhance some cell-mediated immune responses of chickens by modulate macrophages ability. In summary, by manipulating the immune response through the use of different routes, glucan might be used to achieve different effects on the protective immunity to different pathogens.

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