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The Modulating Effect of β-1, 3/1, 6-glucan Supplementation in the Diet on Performance and Immunological Responses of Broiler Chickens*

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ABSTRACT : The object of this trial was to investigate the effect of dietary β-1,3/1,6-glucan supplementation on the performance and immunological response of broiler chickens. Two hundred and forty 1-day old male broilers (39±1 g) were separated into six treatments which were given six different feeds containing 0 (control), 25, 50, 75, 100 and 125 mg/kg dietary β-1,3/1,6-glucan supplementation. On days 21 and 42, body weight gain, feed consumption and feed conversation rate were recorded as measures of growth performance. The levels of key cytokines in the immuno-regulating pathway: interleukin-1 (IL-1), interleukin-2 (IL-2), interferon γ (IFN-γ), tumor necrosis factor α (TNF-α), and the concentrations of signal molecules: peripheral blood plasma globulin, serum Immunoglobulin G (IgG) and intestinal secretary Immunoglobulin A (sIgA), were measured as indices of the immune response to determine suitable levels of dietary β-1,3/1,6-glucan supplementation. The results indicated that performance was elevated quadratically with dietary β-1,3/1,6-glucan supplementation. Maximal growth performance and an enhanced immunological response were obtained at a supplemented level of 50 mg/kg. (**Key Words :** β-1,3/1,6-glucan, Cytokines, Immunoglobulin, Immunomodulating, Broilers)

INTRODUCTION

Antibiotic supplementation is used in the diets of many domestic animals to improve growth, feed efficiency and disease resistance. As a result of the use of antibiotics many pathogenic bacteria have developed resistant strains. This has led to extensive consideration for limiting the use of antibiotics in livestock breeding. Given the demands of both consumers and the policy makers, the development of alternative methods to deal with the problems caused by bacteria in commercial animal production is a high priority for both researchers and farmers.

A number of potential immuno-modulators may serve as antibiotic-alternatives for both the promotion of growth and disease resistance in animal production. β-glucan, which is derived from the cell wall of yeast, bacteria and fungi etc. is a known immuno-modulator in pigs, poultry and some marine animals (Reynolds et al., 1980; Cleary et al., 1999; Sohn et al., 2000; Tzianabos, 2000; Danielle et al., 2002; Suphantharika et al., 2003; Mao et al., 2005; Huff et al., 2006). Variation in the molecular weight, degree of branching, conformation, linkage, and intermolecular association in different plant species results in differing physical properties that affect the biological activity of β-glucans in animals (Bohn and BeMiller, 1995; Kulicke et al., 1997; Pins et al., 2005a, b). An extensive body of literature has shown that the immuno-modulating effects of β-glucan might result in an increase in the functional activity of macrophage and neutrophil cells (Reynolds et al., 1980; Cleary et al., 1999; Tzianabos, 2000; Guo et al., 2003; Yun et al., 2003; Cheng et al., 2004). In recent years, nutritionists have focused on the underlying pathways that regulate or modulate the actions of β-glucan.

With large scale industrial production of β-1,3/1,6-glucan derived from yeast cell walls, this molecule has been identified as a suitable candidate for using as an immuno-modulator in animal rearing. The immuno-protective effects of β-1,3/1,6-glucan have been reported in mammals, fish and birds following dietary supplementation (Mansell et al., 1978; Williams and Di Luzio, 1979; Reynolds et al., 1980; Sakural et al., 1992; Huff et al., 2002, 2006; Guo et al., 2003). Oral administration, or celiac injection of yeast β-1,3/1,6-glucan improves immune function by activating

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Table 1. Ingredient composition of basal diet (as-fed basis)

Ingredients (% of the diet)	0 to 3 weeks	4 to 6 weeks
Corn	56.03	62.38
Soybean meal	37.22	31.47
Soybean oil	2.74	2.47
Calcium hydrogen phosphate dihydrate	1.94	1.65
Limestone	1.17	1.13
L-lysine	0.04	0.08
DL-methionine	0.18	0.15
Antioxidant	0.02	0.02
Sodium chloride	0.35	0.35
50% chloridized choline	0.10	0.10
Vitamin premix[1]	0.02	0.02
Mineral premix[2]	0.20	0.20
β-1,3/1,6-Glucan[3]	0	0
Nutrient level[4]		
Metabolic energy (Mcal/kg)	3.00	3.05
Crude protein (%)	21.00	19.00
Lysine (%)	1.10	1.00
Methionine (%)	0.50	0.45
Calcium (%)	1.00	0.90
Available phosphate (%)	0.45	0.40

[1] Vitamin premix (supplying per kg feed): axerophthol, 12,500 IU; cholecalciferol, 2,500 IU; α-tocopherol, 18.75 mg; menadione, 2.65 mg; thiamine, 2 mg; riboflavin, 6 mg; cobalamin, 25 μg; biotin, 0.0325 mg; folic acid, 1.25 mg; pantothenic acid, 12 mg; niacin, 50 mg.

[2] Mineral premix (supplying per kg feed): Cu, 8 mg; Zn, 75 mg; Fe, 80 mg; Mn, 100 mg; Se, 0.15 mg; I, 0.35 mg.

[3] β-1,3/1,6-Glucan was supplemented additionally as mentioned, 0 mg/kg in the basal diet, 25, 50, 75, 100 and 125 mg in per kg supplemented diets.

[4] Calculated composition.

macrophage cells (Sakural et al., 1992; Guo et al., 2003). Within the poultry industry there has been research focusing on the relationship between supplementation and performance in broilers and laying hens, (Liu et al., 2003; Li et al., 2004), and on immune modulation via dietary supplementation or celiac injection (Huff et al., 2002, 2006; Guo et al., 2003; Liu, et al., 2003). However, the mechanism by which immuno-modulation is achieved remains unknown. Lowry et al. (2005) reported that purified yeast β-1,3/1,6-glucan had bacterial-killing, phagocytosis-stimulating and anti-oxidative-burst functions in four-day-old male Leghorn chickens. In other poultry species, field studies using turkeys (Bahl and Sorgenta, 2002) and a controlled turkey battery study (Huff et al., 2002) have suggested that yeast β-1,3/1,6-glucan may be useful as an alternative to the antibiotics due to its immunomodulating function.

The current trial was conducted to investigate the relationship between dietary β-glucan supplementation and the corresponding immune response in broiler chickens. Given the complexity of the immune system in broilers, it is impossible to measure all physiological parameters of the immune response. We chose to measure the following indices in order to reflect the key regulatory pathways and corresponding immune responses: interleukin-1 (IL-1), interleukin-2 (IL-2), interferon γ (IFN-γ), tumor necrosis factor α (TNF-α), peripheral plasma globulin, serum Immunoglobulin G (IgG) and intestinal secretary Immunoglobulin A (sIgA). The diets of the broilers were supplemented at different levels in order to determine the most effective dosage.

MATERIALS AND METHODS

Chemicals

β-1,3/1,6-glucan was derived from yeast cell walls (*Saccharomyces cerevisiae*), and prepared in the authors laboratory. The chemical content of the β-1,3/1,6-glucan product is: 91.5% glucan, 1.15% crude protein, 0.43% crude fat, while the composition of the remaining 6.92% is unknown. The structure of the β-glucan product was verified by infra-red (IR) and nuclear magnetic resonance (NMR). The polymer unit is given below:

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

The average molecular weight of the product was measured at ~190 KD using the improved specific fluorescence material, calcofluor (Sigma, F3543) method (Rimsten et al., 2003). The β-1,3/1,6-glucan product had no effect on endotoxin production ($p>0.05$) when tested using a commercial endotoxin quantitative kit (Limulus amebocyte lysate, Pyrochrome® quantitative kit, c0060, ACC, Inc., USA).

Animals and diets

Two hundred and forty 1-day old male Arbor Acres broilers (39±1 g) were randomly allotted into six treatments. Each treatment had five replicates (8 broilers in each replicate). Broilers were housed in 3-layer cages and given *ad libitum* access to water and a standard corn-soybean meal type basal experimental diet (Table 1) containing different levels of β-1,3/1,6-glucan supplementation (0, 25, 50, 75, 100 or 125 mg/kg). The diet was formulated in order to meet the National Research Council (NRC, 1994) recommended requirements for all nutrients.

Experimental plan and sample analysis

During the morning of days 21 and 42 of the trial period, body weight and feed consumption were recorded in order to calculate average daily gain (ADG), feed intake and feed/weight gain ratios as indices of growth performance.

In each treatment, 20 blood samples (from four chickens selected randomly in each replicate) were collected from the wing-vein on the left side on days 21 and 42 and centrifuged (2,400 rpm for 20 min) to obtain serum samples. The levels of IL-1, IL-2, IFN-γ and TNF-α, and the concentration of serum IgG were measured using the prepared serum samples.

An additional 20 blood samples were collected using an anticoagulant from the wing-vein on the opposite side and centrifuged (2,400 rpm for 20 min) to obtain plasma samples. The plasma was then stored at -20°C until it could be analyzed for the concentration of peripheral plasma globulin.

Following this the 20 broilers in each treatment were slaughtered and the thymus, spleen and bursa were removed in order to calculate the lymphoid-organ indices as follows:

$$\text{Lymphoid - organ index} = \frac{\text{Weight of lymphoid organ} \times 1.000}{\text{live body weight}}$$

Finally, we also collected small intestinal digesta samples for intestinal sIgA concentration.

The levels of the four cytokines were determined using the double antibody sandwich ELISA method (No.11R015 for IL-1; No.11R020 for IL-2; No.11R030 for TNF-α; No.11R046 for IFN-γ, RapidBio Inc. 23830, Calabaras, CA. 91304). Standards, samples or blanks were analyzed in duplicate. We added 100 μl of diluent containing affinity-purified antibody (Anti-Chook-X, X = IFN-γ, IL-1, IL-2 or TNF-α; concentration = 2 μg/ml for IL-1, 1 μg/ml for the remainder) into each well and incubated the plate overnight at 4°C overnight. After the incubation, aspirated the antibody solution and added 350 μl of washing solution (50 mM Tris, 0.14 M NaCl, 1% Bovine Serum Albumin, 0.05% Tween 20, pH 8.0) into each well and allowed the plate to sit for 30 sec. Following this, we aspirated the washing solution and patted the plate on stacked paper towels until no liquid remained. The wash procedure was repeated five times. We then added 100 μl of the serum samples (the concentration of dilutent was dependant on the results of a pre-trial. The concentrations of the diluent standards were 2,000, 1,000, 500, 250, 125, 62.5, 31.25 or 15.6 pg/ml) into the wells, mixed the contents of the wells for 30 sec, sealed the plates with film-cover and incubated at 37°C for 90 min. The contents of the wells were then aspirated and the plate was washed five times while the incubation was finished. We then added 100 μl of diluent biotin-antibody solution (anti-chook X -Biotin: X = IL-1, IL-2, IFN-γ and TNF-α, concentration = 0.5 μg/ml for IL-1 and IL-2 and 0.25 μg/ml for the latter two) into each well and incubated the film-sealed plate at 37°C for 60 min. Following incubation, the well contents were aspirated and the plate was washed five times using washing solution. We then added 100 μl HRP-streptavidin (concentration = 0.5 μg/ml for IL-1 and IL-2, 0.25 μg/ml for the remaining two) solution into each well and incubated the film-sealed plate at 37°C for 30 min. The HRP-solution was then removed and the plate was washed five times as above. We then transferred 100 μl of previously prepared substrate solution (3,3'5,5-Tetramethylbenzidine (TMB) solution, phosphate-citric acid buffer: TMB = 99:1, V/W, with the addition of 1 μl H_2O_2 per ml of substrate solution prior to use) into each well, mixed the contents of the wells for 10 sec and continued the incubation for 15±10 min. After the final incubation, we added 100 μl stopping solution (2 M H_2SO_4) into each well and shook the plate for 30 sec. The absorbance of the wells was read at 450 nm using a microtiter plate reader (BioRad, Co.). The data from the duplicate wells was averaged.

The concentrations of total protein and albumin in the peripheral plasma were determined using commercial kits (Nanjing Jiancheng Biotech. Co. Ltd, China). The concentration of the plasma globulin was calculated after the value of total protein and the albumin were known.

The concentration of serum IgG concentration was evaluated using a commercially available ELISA kit (Bethyl, Co., 25043 West FM 1097, Montgomery, TX 77356, USA, Catalog No. E30-104). The operating procedure was carried out according to the manufacturers' instructions. Average the duplicate data and find the corresponding value in the standard curve. The intestinal digesta samples were diluted 1:2 (W/V) with the physiological saline and centrifuged at 1,500 rpm for 20 min. The supernatant was then diluted to a suitable concentration (as determined in a pre-trial) as the sIgA sample. The concentration of intestinal sIgA was evaluated using a commercially available ELISA kit (Bethyl, Co., 25043 West FM 1097, Montgomery, TX 77356, USA, Catalog No. E30-103). The operating procedure was carried out according to the manufacturers' instructions.

Statistical analysis

Data were analyzed by ANOVA using the GLM procedure in SPSS (SPSS for Windows, 11.0, SPSS Inc.). Linear and quadratic effects of β-1,3/1,6-glucan supplementation on all indices were determined using the regression program in SPSS 11.0 package. Replicates were used as experimental units for all the experimental indices.

RESULTS

Performance

The average daily gain (ADG) varied quadratically with the dietary supplementation levels of β-1,3/1,6-glucan supplementations (Table 2). Significant increases ($p<0.05$) in body weight were measured in the groups fed with 50 and 75 mg/kg supplements. The remaining treatments (25,

Table 2. Effect of dietary β-1,3/1,6-glucan supplementations on performance of the broilers (Unit of the supplementations was mg/kg, same in the following tables)

	Control	25	50	75	100	125	SEM	p-value (Quadratic)
ADG (g)								
0 to 3 wk	31[a]	32[a]	36[b]	35[b]	33[a]	32[a]	1.94	0.038
4 to 6 wk	51[a]	54[a]	59[b]	58[b]	53[a]	51[a]	3.45	0.026
Feed intake (kg)								
0 to 3 wk	0.85[a]	0.88[a]	0.96[b]	0.95[b]	0.91[a]	0.91[a]	0.04	0.041
4 to 6 wk	1.82[a]	1.90[a]	2.11[b]	2.03[b]	1.89[a]	1.82[a]	0.12	0.011
Feed/weight gain								
0 to 3 wk	1.24[a]	1.18[a]	1.17[ab]	1.07[b]	1.22[a]	1.24[a]	0.069	0.013
4 to 6 wk	1.84[a]	1.83[a]	1.72[b]	1.71[b]	1.78[a]	1.79[a]	0.056	0.021

* A similar superscript indicates no significant difference ($p>0.05$); a different superscript indicates a significant difference ($p<0.05$).

Table 3. Peripheral blood serum IL-1, IL-2, IFN-γ and TNF-α levels (pg/ml)*

	Control	25	50	75	100	125	SEM	p-value (Quadratic)
IL-1 levels								
d 21	0.142[a]	0.150[a]	0.152[ab]	0.155[b]	0.147[a]	0.143[a]	0.005	0.023
d 42	0.150[a]	0.188[b]	0.190[b]	0.158[a]	0.151[a]	0.152[a]	0.021	0.010
IL-2 levels								
d 21	0.473[a]	3.756[c]	3.779[c]	4.763[c]	2.547[c]	2.442[c]	1.745	<0.000
d 42	3.619[a]	6.754[c]	8.136[c]	9.997[c]	7.511[c]	6.255[bc]	2.125	<0.000
IFN-γ levels								
d 21	0.115[a]	0.128[a]	0.212[c]	0.165[b]	0.134[ab]	0.121[a]	0.037	0.015
d 42	0.113[a]	0.131[a]	0.137[b]	0.133[ab]	0.122[a]	0.119[a]	0.009	0.038
TNF-α levels								
d 21	15.735[a]	17.615[a]	20.987[b]	22.905[b]	18.816[b]	12.186[a]	3.814	0.022
d 42	13.570[b]	16.875[b]	25.189[c]	22.514[c]	17.800[b]	9.474[a]	5.737	0.015

* A similar superscript indicates no significant difference ($p>0.05$); a different superscript indicates a significant difference ($p<0.05$).

Table 4. Effect of dietary β-1,3/1,6-glucan supplementations on lymphoid-organ indices of the broilers*

	Control	25	50	75	100	125	SEM	p value (Quadratic)
Thymus index								
0 to 3 wk	2.28[a]	2.41[a]	3.09[c]	2.97[c]	2.73[bc]	2.63[b]	0.071	0.000
4 to 6 wk	1.59[a]	1.88[ab]	2.49[e]	2.41[e]	2.35[bc]	2.18[b]	0.116	0.000
Bursa index								
0 to 3 wk	2.08[a]	2.34[ab]	2.75[bc]	3.08[c]	2.56[ab]	2.55[ab]	0.081	0.003
4 to 6 wk	1.19[a]	1.45[ab]	1.79[b]	1.71[b]	1.72[b]	1.27[a]	0.049	0.003
Spleen index								
0 to 3 wk	1.22[a]	1.79[d]	1.83[d]	1.71[cd]	1.48[bc]	1.34[ab]	0.0050	0.000
4 to 6 wk	1.74[a]	2.10[b]	2.51[d]	2.41[cd]	2.13[bc]	2.18[b]	0.054	0.000

* A similar superscript indicates no significant difference ($p>0.05$); a different superscript indicates a significant difference ($p<0.05$).

100 and 125 mg/kg), while numerically heavier, were not significantly ($p>0.05$) heavier than the control animals. A significant increase ($p<0.05$) in feed intake was also observed in the groups receiving 50 and 75 mg/kg supplementation. As a result these two groups had significantly lower feed/weight gain ratios ($p<0.05$) than the control group.

Levels of IL-1, IL-2, IFN-γ and TNF-α in serum

The levels of the four cytokines: IL-1, IL-2, IFN-γ and TNF-α in the serum are shown in Table 3. As with ADG we observed a quadratic increase in cytokine levels following β-1,3/1,6-glucan supplementation ($p<0.05$). Higher levels were measured in the group supplemented with 50 and 75 mg/kg β-1,3/1,6-glucan than in the other groups. However, IL-2 levels were significantly higher ($p<0.01$) in all supplemented groups.

Lymphoid-organ indices

Results of lymphoid-organ indice analysis are shown in Table 4. We found a very significant ($p<0.01$) increase in all three lymphoid-organ indices when the animals were supplemented with β-1,3/1,6-glucan. The only exception was for the bursa index, which was not significantly

Table 5. Peripheral blood plasma globulin, serum IgG & intestinal sIgA concentration (mg/L)*

	Control	25	50	75	100	125	SEM	p value (Quadratic)
Plasma globulin								
d 21	1,328.72[a]	1,516.54[b]	1,810.56[c]	1,857.79[c]	1,676.74[c]	1,523.34[c]	32.30	0.000
d 42	1,411.75[a]	1,611.82[b]	1,824.71[c]	1,890.12[c]	1,697.35[c]	1,561.42[ab]	37.30	0.000
Serum IgG								
d 21	530.15[a]	726.84[b]	742.91[b]	806.13[b]	675.92[b]	544.09[a]	111.84	0.013
d 42	799.62[a]	1,164.42[b]	1,286.25[b]	1,415.43[b]	889.44[a]	826.53[a]	260.71	0.007
Intestinal sIgA								
d 21	53.05[a]	102.96[b]	156.38[e]	137.84[d]	128.52[cd]	121.76[c]	6.98	0.000
d 42	59.90[a]	111.17[b]	173.02[d]	139.26[c]	130.06[c]	122.39[bc]	7.38	0.000

* A similar superscript indicates no significant difference ($p<0.05$); a different superscript indicates a significant difference ($p<0.05$).

different ($p>0.05$) for the group fed with 125 mg/kg supplemented diet on day 42.

Concentrations of peripheral plasma globulin, serum IgG and intestinal sIgA

The concentrations of peripheral plasma globulin, serum IgG and intestinal sIgA are shown in Table 5. As with the lymphoid-organ indices, we found significant ($p<0.05$), and very significant differences ($p<0.01$) in the levels of peripheral plasma globulin following supplementation with β-1,3/1,6-glucan.

We observed a similar pattern with serum IgG concentrations. Significantly higher ($p<0.05$) concentrations of IgG were measured on both day 21 and day 42 in all treatment groups with the exception of the group fed on a 125 mg/kg supplemented diet.

Similarly, the concentrations of intestinal sIgA were significantly elevated in all treatment groups on both day 21 and 42 ($p<0.01$).

DISCUSSION

An improvement in ADG and a decline in the feed/weight gain ratios were observed in chickens fed β-1,3/1,6-glucan- supplemented diets. Our findings are similar to that of Liu et al. (2003), who reported that a significant improvement in the performance of broilers was obtained when yeast β-1,3/1,6-glucan was supplemented in their diet at a level of 50 mg/kg. Researchers have reported a decrease in performance following supplementation with β-glucan in trout and pigs. The authors hypothesized that the decrease in performance resulting from β-glucan supplementation was attributable to decreased intake of nutrients and efficiency of nutrient utilization for growth. Despite these reports the majority of research has shown that β-glucan results in improved performance (Tokunaka et al., 2000; Thanardkit et al., 2002, Li et al., 2003). A review of the literature suggests that the optimum level of β-glucan supplementation in order to maximize animal performance is between 25 and 200 mg/kg when fed throughout the entire feeding period. For broilers, a more suitable supplementation level is 25 to 150 mg/kg. Further research is required to determine the optimum supplementation level of β-1,3/1,6-glucan in order to achieve greatest performance. Structure, molecular weight and the origin of the β-1,3/1,6-glucan are all factors that can alter the effectiveness of supplementation.

The differences we measured in levels of IL-1, IL-2, IFN-γ and TNF-α in the peripheral serum most likely resulted from a quadratic dosage response to the dietary supplementation levels of β-1,3/1,6-glucan. We hypothesize that β-1,3/1,6-glucan may join with the MHC compound and subsequently activate monocytes or macrophages. This action leads to the subsequent immuno-potentiation. Immuno-potentiation may include the activation of cytotoxic macrophages, T-helper and natural killer cells, the promotion of T-cell proliferation and differentiation, and the activation of the alternative complement pathway (Bohn and BeMiller, 1995). Within the regulatory pathways described above, IL-1, IL-2, IFN-γ and TNF-α may act as signal-messengers in the immuno-regulating network. Levels of these molecules changed dosage-dependently in the current study corresponding to the levels of β-1,3/1,6-glucan supplementation. As a result of these changes in concentration the signal that is transferred to the effecting molecule-immunoglobulins, or to the immuno-organs and tissues is also altered. Our measurements of IL-1 and TNF-α following supplementation were partly consistent with the work of Vetvicka (2004) on mice. In that study the levels of IL-1 and TNF-α increased after the injection of lentinan (a form of β-Glucan). An *in vitro* experiment using the mouse macrophage cell line, RAW264.7, also illustrated that the secretion of the TNF-α increases as a result stimulated with zymosan (a form of yeast β-glucan) (Young et al., 2001). Furthermore, an *in vitro* experiment by Guo et al. (2003) showed that β-1,3/1,6-glucan stimulates celiac macrophages to secrete IL-1 as well as inducing macrophages to proliferate in culture. During immune stimulation, the most prominent process in the response of an animal is to release cytokines such as IL-1, TNF-α and IL-6. The release of

these cytokines stimulates or modulates the immune response in the animal body and initiates the formation of stress proteins. In the current study, β-1,3/1,6-glucan acted as both a stimulating and effecting molecule in the regulatory cycle of the macrophage. The levels of IFN-γ and IL-2 may also be actively influenced by feedback from the macrophages. The changes in cytokine levels increase the preparedness of the body to respond to internal challenges.

At some levels, β-1,3/1,6-glucan supplementation may also decrease the release of cytokines (Tzianabos, 2000). In that study it was suggested that the animal utilized more nutrients for tissue growth at the expense of immune responses when stimulated with different levels of β-glucan supplementation. That result might explain the improved growth response to the lower β-glucan level supplementation observed by Dritz et al. (1995) and Schoenherr (1995). This effect is often referred to as stimulus-resistance. The stimulus-resistance effect of β-glucan can be attributed to three possible mechanisms. These include: inhibition of the synthesis of cytokines, such as IL-10 (Hoqaboam et al., 1998), promotion of cytokine receptor secretion (Poutsiaka et al., 1993), and release of macrophage arachidonic acid metabolism (PGE) in response to soluble β-glucan (Castro et al., 1994). In the current trail, the fact that supplementation with 25, 100 or 125 mg/kg β-1,3/1,6-glucan did not produce the same level of enhancement as 50 or 75 mg/kg may be due to stimulus resistance. However, the mechanisms underlying the interaction between the level of β-1,3/1,6-glucan supplementation and the levels of cytokines are not clear at this point.

The lymphoid-organ indices measured in the current study provide further evidence that β-1,3/1,6-glucan has an immuno-modulating function. Both on day 21 and 42, the lymphoid organ indices were significantly elevated in correlation with the level of dietary β-1,3/1,6-glucan supplementation. These results are supported by Liu et al. (2003), who reported significant improvement in broilers performance when β-1,3/1,6-glucan was supplemented at a level of 50 mg/kg. This may provide an alternate explanation for the change in cytokine levels as production is likely to increase due to the presence of greater numbers of that might prove the changing trends of the levels of the cytokines, IL-1, IL-2 and IFN-γ from another side. More cytokines would be secreted from the increased lymphocytes and macrophages.

The lower effecting molecules, IgG and sIgA are regulated by the concentration of cytokines, the numbers of lymphocytes and exterior stimulating molecules. In this instance β-1,3/1,6-glucan most likely affects both cellular and humoral immunity. The effecting molecule of humoral immunity, immunoglobulin, responds to the upper signal-delivery. In the current study, the changes in concentration of the peripheral plasma globulin, serum IgG and intestinal sIgA actually represent the response of B lymphocytes to stimulation from IL-2 or cytotoxic T cells (CTL). The changes in concentration of the immunoglobulins may also explain the changes in the levels of the corresponding cytokines. However, the mechanism underlying the relationship between β-1,3/1,6-glucan supplementation and the concentrations of the immunoglobulin are not yet clear.

The concentration of peripheral plasma globulin is an indicator of the response to exterior stimulation. In this experiment, the increase in the concentration of peripheral plasma globulin was most likely the result of stimulation by dietary β-1,3/1,6-glucan supplementation.

To enhance modulated immunity, a suitable concentration of β-1,3/1,6-glucan is needed by the animal. Both our study and that of Liu et al. (2003) indicate that 50 mg/kg of dietary β-1,3/1,6-glucan is an acceptable level for supplementation. This is similar to the level recorded for pigs (Wang et al., 2006).

The intestinal mucosal is an important system which can maintain the balance of the intestinal environment, protect the intestinal tract from being damaged by the bacterial over-population, and insure the absorption of nutrients by the intestinal epithelia. Immuno-protective function is provided by sIgA (Ramaswamyk et al., 1996; Williams et al., 1998). In the current study, we hypothesize that β-1,3/1,6-glucan stimulates the gut associated lymphoid tissue (GALT) to secrete more sIgA in order to enhance mucosal immunological function. Gordon et al. (2001), reported the existence of a β-glucan receptor, dectin-1, located on macrophages, lymphocytes and other blood cells. The binding of β-glucan to the receptor may cause macrophage, lymphocyte or other blood cells to secrete greater quantities of lower signal molecules. Accordingly, sIgA production might be enhanced in this manner and secreted by the intestinal macrophages and lamina propria lymphocytes. Intestinal sIgA secretion can also be determined by measuring the levels of the cytokines, IL-2 and IFN-γ (Kramer et al., 1995). In the current study, we suggest that the increase in levels of serum cytokines may provide another mechanism for increasing the secretion of sIgA. Given that sIgA has anti-bacterial and anti-infectious functions (Ai et al., 2000), intestinal sIgA improves the integrity of the intestinal tract and ensures more nutrients can be absorbed by the intestinal epithelia, thus allowing for better growth and performance.

Modulation of the immune system depends on the joint function of cytokines, plasma globulin, serum IgG and intestinal sIgA. Given our results we suggest that β-1,3/1,6-glucan may play a role in the initiation of immune modulation. Therefore, suitable supplementation would be

beneficial to the performance of broiler chickens.

In conclusion, β-1,3/1,6-glucan supplementation has a positive effect on modulating the animals interior environment by maintaining increased levels of cytokines and immunoglobulins. In addition, supplementation increases performance by improving ADG and reducing the feed/weight gain ratio. β-1,3/1,6-glucan supplementation has the potential to improve the immune function of broiler chickens fed in conventional environments.

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