Asian-Aust. J. Anim. Sci.
Vol. 21, No. 7 : 1027 - 1032
July 2008

www.ajas.info

Growth Performance and Antibody Response of Broiler Chicks Fed Yeast Derived β-Glucan and Single-strain Probiotics

B. K. An, B. L. Cho, S. J. You, H. D. Paik, H. I. Chang[1], S. W. Kim[2], C. W. Yun[1] and C. W. Kang*

College of Animal Bioscience and Technology, Konkuk University
1 Hwayang-dong, Gwangjin-gu, Seoul 143-701, Korea

ABSTRACT : A study was conducted to evaluate the effects of dietary yeast derived β-glucan and single-strain probiotics on the growth performance and antibody response in broiler chicks. Six hundred and thirty 1-d-old male broiler chicks were divided into seven groups, placed into three pens per group (30 birds per pen) and fed one of seven non-medicated corn-SBM based experimental diets containing 0.025, 0.05 or 0.1% *Saccharomyces cerevisiae* β-glucan and 0.05, 0.1 or 0.2% *Bacillus amyloliquefaciens* (BA-pro, 1.3×10^9/g) or devoid of them for 5 wk. The body weight gains in groups fed diets containing 0.025 or 0.1% β-glucan, 0.1% or 0.2% BA-pro were significantly higher ($p<0.05$) than the control over 1-35 d. Feed conversion rates of groups fed β-glucan and BA-pro tended to be improved compared to the control group. There were no significant differences in the relative weights of liver, abdominal fat and breast muscle. No significant differences were observed in the activities of serum enzymes and concentrations of various cholesterol fractions. The antibody titers against Newcastle disease or infectious bronchitis virus in the chicks fed diets containing β-glucan and BA-pro were significantly higher ($p<0.05$) than in the control. The concentrations of cecal lactic acid bacteria in all groups fed BA-pro were significantly increased ($p<0.05$) compared to the control. These results indicated that dietary yeast derived β-glucan and BA-pro exerted growth-promoting and immune-enhancing effects in broiler chickens. In addition, BA-pro added to the diets modulated the profiles of cecal microflora, reflecting a potential to be beneficial microorganisms in chickens. (**Key Words :** Yeast Derived β-Glucan, Single-strain Probiotics, Growth Performance, Antibody Response, Cecal Microflora, Broiler Chickens)

INTRODUCTION

The extensive uses of subtherapeutic antibiotics to improve growth performance and prevent intestine from infectious diseases have led to problem of drug residues in animal products and emerge of new antibiotic-resistance bacteria. In this regard, the routine use of antibiotics in animal feed was less common and endeavors are made to develop alternate means for preventing and treating infectious disease in poultry industry. Probiotics and β-glucan are increasingly being used in poultry feed, in place of antibiotics, as alternative means to prevent intestine from infectious diseases and modulate of immune responses, respectively (Sohn et al., 2000).

* Corresponding Author: C. W. Kang. Tel: +82-2-450-3669, Fax: +82-2-452-9946, E-mail: kkucwkang@empal.com
[1] School of Life Science and Biotechnology, Korea University, Seoul 136-701, Korea.
[2] Department of Chemical and Biological Engineering, Korea University, Seoul 136-701, Korea.
Received October 2, 2007; Accepted December 2, 2007

Probiotics have been defined as 'live microbial feed supplement that benefits the host animal by improving its intestinal microflora balance' (Fuller, 1989). In previous studies, *Lactobacilli* and *Bacillus subtilis* administration resulted in improve growth performance and feed efficiency (Jin et al., 1996; Mohan et al., 1996) and stimulate the production of natural antibodies in chickens (Haghighi et al., 2006). Moreover, it has been shown that *Lactobacilli* and *E. faecalis* could protected chickens against pathogens by colonization in the gastrointestinal tract (Nisbet et al., 1993). β-Glucan derived from a variety of yeast cell wall have been suggested to modulate both specific and non-specific immune responses in various animals (Chae et al., 2006; Eicher et al., 2006). Dietary β-glucan has been shown to have beneficial effects on the functional activity of macrophage and heterophils (Lowry et al., 2005), and release some kind of cytokines after an immunological challenge (Li et al., 2005). Cheng et al. (2004) also reported that relatively high levels of dietary β-glucan enhanced some cellular immune responses of chickens by modulate

Table 1. Composition of the experimental diets

Ingredients	Starter	Finisher
Yellow corn	56.60	60.88
Soybean meal	30.21	28.39
Corn gluten meal	5.00	3.18
Tallow	4.04	4.25
Vit.+Min. mixture[1]	0.15	0.15
L-lysine HCl (98%)	0.18	-
DL-methionine (99%)	0.16	0.04
Dicalcium phosphate	2.00	1.50
Limestone	1.07	1.12
Choline-Cl (50%)	0.11	0.04
Salt	0.38	0.35
Anticoccidial	0.10	0.10
Total	100	100
Calculated values		
TMEn (kcal/kg)	3,050	3,100
Crude protein (%)	21.5	19.5
Ca (%)	1.00	0.90
Available P (%)	0.45	0.36
Lysine (%)	1.20	1.00
Total TSAA (%)	0.90	0.73

[1] Vit.+Min. = Vitamin plus mineral mixture provided the following nutrients per kg of diet: vitamin A, 18,000 IU; vitamin D_3, 3,750 IU; vitamin E, 30 IU; vitamin K_3, 2.7 mg; vitamin B_1, 3.0 mg; vitamin B_2, 9.0 mg; vitamin B_6, 4.5 mg; vitamin B_{12}, 30.0 μg; niacin, 37.5 mg; pantothenic acid, 15 mg; folic acid, 1.5 mg; biotin, 0.07 mg; Fe, 75.0 mg; Zn, 97.5 mg; Mn, 97.5 mg; Cu, 7.5 mg; I, 1.5 mg; Se, 0.2 mg.

macrophage chemotaxis activity, without growth promoting effect.

The objective of the present study was to investigate the dietary effects of β-glucan and single-strain probiotics on growth performance, humoral immunity and the cecal microflora in broiler chicks.

MATERIALS AND METHODS

A total of six hundred thirty Ross male broiler chicks at 1 d of age were weighed and randomly assigned into seven experimental treatments. Each treatment was comprised three replicates of 30 birds. The chicks were fed one of seven non-medicated corn-SBM based diets for 5 wks; a control diet containing neither β-glucan nor probiotics, 3 diets containing β-glucan at different dosages (0.025, 0.05 or 0.1%, respectively), and 3 diets containing probiotics at different dosages (0.05, 0.1 or 0.2%, respectively). The β-glucan used in this study was isolated from cell mass by wild-type *Saccharomyces cerevisiae* JUL3 and provided by School of Life Science and Biotechnology of Korea University. The *Bacillus amyloliquefaciens* KU801 strain (BA-pro) was provided by College of Bioscience and Technology of Konkuk University and contained 1.3×10^9 living microorganisms/g.

The non-medicated experimental diets were purchased from a commercial feed manufacture and mixed with the doses of β-glucan and BA-pro. The chicks were allowed to have free access to a starter diet during first 3 wks and continually to a finisher diet during second 2 wks and free access to water. Nutrients and energy concentrations were met or exceeded minimum requirements of NRC (1994). The formula and chemical compositions of experimental diets are shown in Table 1. Animal facilities and husbandry were similar to conditions described by An et al. (1995). The chicks were initially started at 33°C; the temperature was gradually decreased by 4°C weekly to 22°C by the end of 3 wk. Lighting was kept at 23/1 h light/dark cycle throughout the experimental period. Body weight and feed intake on a pen basis were weighed weekly and feed conversion rate was also calculated.

Antibody production to live Newcastle disease virus (NDV) and infectious bronchitis virus (IBV) vaccines were used to examine the humoral immune response of chicks fed β-glucan and probiotics. Chicks were inoculated commercially available live NDV and IBV vaccines by intramuscular injection on 2 wk of experiment and blood were taken from the jugular veins 2 wk of after injection. Serum samples were analyzed for anti-NDV and anti-IBV antibody titers by ELIZA with commercial kits, following the manufacturer's direction (IDDEX Laboratory, Inc., ME).

At the end of the experimental period, nine chicks from each treatment were selected and weighed individually. The blood was drawn from the jugular vein using a syringe for determination of the concentration of various lipid fractions and components. At necropsy, the liver, abdominal fat, spleen and right breast muscle were immediately removed and weighed. The serum was separated from each blood sample by centrifugation and stored at -30°C until use. The concentration of total cholesterol (Total-C) and high-density lipoprotein cholesterol (HDL-C), the activity of glutamic-oxaloacetic transaminase (GOT) and glutamic-pyruvic transaminase (GPT) in serum were measured according to the colorimetric method using cholesterol diagnostic-kit (Cholesterol E kit and HDL-cholesterol kit, Youngdong Medical Corporation) and GOT-GPT assay kit (GOT-GPT assay kit, Youngdong Medical Corporation).

The cecal digesta homogenates in PBS were serially diluted from 10^{-1} to 10^{-7}. Dilutions were subsequently plated on duplicate selective agar media for enumeration of target bacterial strains. Total microbes, *coliforms* and *Lactobacillus* spp. were enumerated using nutrient agar, MacConkey agar, and MRS agar, respectively, using the traditional method (Tuohy et al., 2002). Each plate was incubated at 37°C, for 24 to 72 h anaerobically or aerobically, and colonies were then counted. Results obtained were presented as base-10 logarithm colony-forming units per gram of cecal digesta.

The main effects between treated groups were subjected

Table 2. Dietary effects of β-glucan and probiotics on growth performances in broiler chickens[1]

	Control	β-Glucan			Probiotics			Pooled SEM
		0.025%	0.05%	0.1%	0.05%	0.1%	0.2%	
Initial BW (g/bird)	46.77	46.80	46.79	46.79	46.79	46.79	46.80	0.50
Final BW (g/bird)	1,577.3	1,722.7	1,672.8	1,721.6	1,699.7	1,752.4	1,711.7	15.9
BW gain (g/day/bird)								
1-21 d	35.35	35.94	35.98	37.28	36.38	36.69	36.99	0.30
22-35 d	74.22	82.47	78.62	82.95	80.15	83.80	81.95	0.95
1-35 d	54.79[b]	59.21[a]	57.30[ab]	60.12[a]	58.26[ab]	60.25[a]	59.25[a]	0.50
Feed intake (g/day/bird)								
1-21 d	59.4	60.0	58.8	59.6	59.6	59.6	60.0	0.2
22-35 d	143.3	148.1	147.5	146.5	142.6	141.9	143.2	1.4
1-35 d	97.0	99.1	98.2	98.6	96.5	96.2	97.1	0.6
Feed/gain								
1-21 d	1.72	1.67	1.63	1.60	1.64	1.63	1.64	0.01
22-35 d	1.94	1.69	1.88	1.77	1.78	1.69	1.75	0.03
1-35 d	1.80	1.68	1.80	1.71	1.73	1.67	1.72	0.02

[1] Values are presented Mean±SE.

[a, b] Values with different superscripts were significantly different ($p<0.05$).

Table 3. Dietary effects of β-glucan and probiotics on carcass characteristics and blood profiles in broiler chickens[1,2]

	Control	β-Glucan			Probiotics			Pooled SEM
		0.025%	0.05%	0.1%	0.05%	0.1%	0.2%	
Liver (g/100 g BW)	2.00	1.94	1.95	1.95	1.99	2.04	1.97	0.03
Abdominal fat (g/100 g BW)	1.87	1.68	1.56	1.61	1.37	1.64	1.64	0.05
Breast muscle (g/100 g BW)	6.38	6.28	6.61	6.57	6.13	5.88	6.43	0.09
Spleen (g/100 g BW)	0.11	0.11	0.10	0.09	0.10	0.11	0.10	0.01
GOT (U/100 ml)	279.80	267.51	270.02	266.46	266.73	264.65	249.60	2.34
GPT (U/100 ml)	9.12	9.34	10.99	10.84	9.80	9.14	10.60	0.42
Total-C (mg/100 ml)	121.02	126.74	130.70	148.04	119.70	124.60	129.18	3.01
HDL-C (mg/100 ml)	63.85	63.69	67.05	70.01	64.74	63.41	63.37	1.31
HDL-C/total-C	0.53	0.50	0.51	0.48	0.54	0.51	0.50	0.01

[1] GOT = Glutamic-oxaloacetic transaminase; GPT = Glutamic-pyruvic transaminase; Total-C = Total cholesterol; HDL-C = High density lipoprotein-cholesterol.

[2] Values are presented mean±SE.

to ANOVA using the general linear models procedure of SAS (2002), and significant differences were determined using Duncan's multiple range test at the level of $p<0.05$ (Duncan, 1955). Percentage data were transformed to arc sine percentages before square root percentages ANOVA was performed.

RESULTS AND DISCUSSION

Growth performance and carcass characteristics

The body weight (BW) gain, feed intake and feed/gain in groups fed the experimental diets are shown in Table 2. The treated broiler chicks exhibited higher growth rates than the control bird in terms of final BW and BW gain. No significant differences in the growth performance were observed among the dietary treated groups. BW gain in chicks fed diet containing 0.025 or 0.1% β-glucan and 0.1 or 0.2% BA-pro was significantly higher ($p<0.05$) as compared with that of control in total experimental period. BW gain during the first 3 wks and second 2 wks tended be increased in chicks fed diets containing β-glucan or BA-pro. There were no significant differences in feed intake during the first 3 wks, second 2 wks and total period. Feed/gain in the groups fed β-glucan or BA-pro was superior to that of control, but not significantly. No significant differences were also observed in relative weights of liver, abdominal fat, breast muscle and spleen (g/100 g body weight) as shown in Table 3.

A number of studies with broiler chickens have reported that feeding of *Lactobacilli* and *Bacillus subtilis* improved BW gain and feed conversion rate (Jin et al., 1996; Mohan et al., 1996). However, some studies have shown that *L. acidophilus* and *S. faecium* exerted no positive effects on growth performance (Maiolino et al., 1992). Khaksefidi and Rahimi (2005) reported that feeding of multi-species probiotics increased BW gain and feed conversion rate in finisher period. Consistent improvement in growth parameters in chicks fed *L. casei* and *L. acidophilus* have also reported by Huang et al. (2004). Edens et al. (1997) showed that feeding of *L. reuteri* resulted in a higher villus

Table 4. Dietary effects of β-glucan and probiotics on anibody titers against NDV and IBV and cecal microflora profiles in broiler chickens[1,2]

	Control	β-Glucan			Probiotics			Pooled SEM
		0.025%	0.05%	0.1%	0.05%	0.1%	0.2%	
NDV titer (log)	3.75[b]	5.12[ab]	6.00[a]	6.13[a]	5.38[a]	5.75[a]	6.00[a]	0.21
IBV titer (log)	3.62[c]	5.00[b]	5.88[ab]	6.50[a]	5.88[ab]	6.00[ab]	5.63[ab]	0.18
Total microbes (log cfu/g)	6.88	6.25	6.50	6.88	6.38	6.50	6.38	0.18
Coli. forms (log cfu/g)	4.25	4.25	4.38	4.13	4.13	4.00	4.00	0.20
Lactic acid bacteria (log cfu/g)	4.75[b]	5.63[ab]	5.50[ab]	5.62[ab]	6.50[a]	6.75[a]	6.63[a]	0.16

[1] NDV = Newcastle disease virus; IBV = Infectious bronchitis virus. [2] Values are presented mean±SE.
[a, b, c] Values with different superscripts were significantly different ($p<0.05$).

height, indicating that probiotics was potentially able to enhance nutrients absorption and thereby improve growth performance. Hiss and Sauerwein (2003) reported that feeding of 0.015 or 0.03% β-glucan resulted in a numerical increase in average daily gain in postweaning piglets. Recently, Huff et al. (2006) also found that 7 d feeding of β-glucan product prior to *E. coli* challenge improved BW gain and feed conversion rate in broiler chickens as compared with unfed challenged control.

A higher level of probiotics does not always lead to the better production parameters. Panda et al. (2000) found that supplementing multi-strain probiotics of 100 mg/kg diet improved egg production as compared with those of unfed control and a higher dosage group. In contrast, Senanl et al. (1997) reported that a higher level of *L. casei* performed better than a lower level in terms of BW gain. In present study, BW gain in the birds fed higher levels of BA-pro (0.1 or 0.2%) was significantly higher ($p<0.05$) than control in 1-35 d, and it means that BA-pro can be used for broiler feed up to 0.2% without any negative effects on growth performance.

Blood profiles

The concentrations of various lipid fractions and components of serum in chicks fed the experimental diets are shown in Table 3. No significant differences were also observed in the concentrations of Total-C and HDL-C, and the ratio of HDL-C/Total-C. The dietary treatments did not have significant effects on the activities of GOT and GPT. Probiotics supplementation has been reported to frequently lower blood cholesterol in experimental animals (Liong and Shah, 2006). Jin et al. (1998) have been suggested that feeding of *Lactobacillus* culture significantly lowered the level of serum cholesterol in broiler chickens. However, there have been contradictory findings in relation to the level of blood cholesterol. Grunewald and Mitchell (1983) reported that *L. acidophilius* failed to lower blood cholesterol in rat. Therefore, it is likely that hypocholesterolemic effect by dietary probiotics does not always occur because differences in the probiotics strains, optimum dose, feeding frequency, and duration of treatments. In this study, the concentrations of total cholesterol and HDL-C, and the ratio of HDL-C/Total-C were not also influenced by dietary β-glucan.

Antibody production

The antibody titers against NDV and IBV in the chicks fed the experimental diets are presented in Table 4. The levels of NDV titer were significantly higher ($p<0.05$) in all the treated groups, except for 0.025% β-gucan than that of control. The dietary supplementation of β-gucan and BA-pro resulted in significant increase ($p<0.05$) in the antibody titer against IBV as compared with that of control. The level of IBV titer in group fed 0.1% β-gucan was highest and significantly higher ($p<0.05$) than those of control and 0.025% β-gucan group. The result agreed with Huang et al. (2004) who previously reported that probiotics could modulate the systemic antibody response to antigens in chickens. Haghighi et al. (2006) also found that birds fed *L. acidophilus* and *B. bifidum* had significantly more serum antibody to SRBC than that of unfed control. Dietary β-gucan has been also shown to improve the humoral immune response of pigs and modulate cell-mediated immune response by enhancing the increase of cytokines after an immunological challenge (Li et al., 2005). Cheng et al. (2004) suggested that β-gucan feeding enhanced some cell-mediated immune responses of broiler chickens by modulating macrophage activity. It can be postulated that some of these effects are mediated by cytokines secreted by immune cells stimulated with β-gucan. In this study, feeding of β-gucan and BA-pro resulted in significant increase ($p<0.05$) in the antibody titer against NDV and IBV than those of unfed control. It was suggested that dietary β-gucan and BA-pro might be useful in treating against viral diseases because of immunostimulating activity.

Cecal microbial population

The concentration of cecal lactic acid bacteria was significantly increased ($p<0.05$) in the groups fed diets containing BA-pro than that of control, whereas the levels of total microbes and *coli* form bacteria were not changed by the dietary β-gucan and BA-pro (Table 4). Feeding BA-pro resulted in a beneficial modulation of cecal microflora

as evidenced by the numerous increases in the concentration of lactic acid bacteria. These results concur with those of Mountzouris et al. (2007) who found that broilers fed multi-species probiotics had higher numbers of *Lactobacillus* spp. in cecal microflora. It is not known why the supplementation of *Bacillus* strain failed to reduce the number of total microbes and *coli* form bacteria in cecal content.

In conclusion, dietary yeast derived β-glucan and BA-pro exerted growth-promoting and immune-enhancing effects in broiler chickens fed non-medicated diet. In addition, BA-pro added to the diets positively modulated the profiles of cecal microflora, resulting in a significant probiotic effect.

ACKNOWLEDGMENT

The financial support by Biogreen 21 (2005040103-47981870300) through the Rural Development Administration is gratefully acknowledged.

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