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Effects of dietary supplementation with different levels and molecular weights of fungal β-glucan on performances, health and meat quality in broilers

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Objective: To investigate the effects of dietary supplementation with different levels and molecular weights of fungal β-glucan on productive performances, health, carcass traits and meat quality in broilers.
Methods: Two hundred and ten of one-day-old chicks with equal sex were assigned to seven experimental groups in 2×4 factorial arrangement. These groups were supplemented with (0, 10, 30, and 60 ppm) of molecular weight 1-3, 1-6 β-glucan (low or high). High molecular weight β-glucan (H: 943 kDa) was obtained from *Ophiocordyceps dipterigena* BCC 2073, whereas H with γ-Irradiation treatment was performed to achieve low molecular weight β-glucan (L: 8 kDa).
Results: There was no statistical significance in productive performances, apparent digestibility and interaction between fixed factors along 42 days of experiment ($p>0.05$). A higher caecal amylase activity was present in the group that received L, while there was a dramatic decrease in H and the control groups, respectively ($p<0.05$). The increase of supplemental dose increased caecal amylase activity ($p<0.05$). Immunomodulatory effects from L was revealed by the marked increase of phagocytic activity, relative weight of thymus and bursa of fabricius ($p<0.05$). Similarly, the additive dose at 30 ppm provided the same results, whereas the only significant difference with supplementation at 60 ppm was an increase in phagocytic activity ($p<0.05$). Interestingly, villi height of broilers fed L was higher than other groups ($p<0.05$). The treatments did not influence haematology, blood chemistry, antibody production level against vaccination, carcass traits and meat quality ($p>0.05$).
Conclusion: The supplementation of L at 30 ppm was suggested to achieve benefits of immune modulation without adverse effects on other parameters.

Keywords: Amylase; Bursa of Fabricius; Digestive Enzyme Activity; Immunity; *Ophiocordyceps dipterigena*; Phagocytosis

INTRODUCTION

The ban of using antibiotics as growth promoters was announced in several countries to reduce the problems of bacterial resistance and antibiotic residue in animal products. A deterioration of animals' performance, health and products were observed after this regulation [1]. Therefore, several techniques have been studied to solve this problem. Researchers have studied dietary supplements with growth promoters and immunomodulation properties in an attempt to solve this problem.

β-Glucan is non-starch polysaccharide which occurs in cell structure of cereal crops (mainly barley and oat), yeasts, mushrooms and molds [1-3]. The diverse chemical structure of β-glucan is reported as depending on the origin which influences the physiological response after the usage. β-(1→3)-linkages are the basic structure of β-glucan, whereas β-(1→4)-linkages and β-(1→6)-linkages are found only in plants and fungi, respectively [3]. The intake of (1→3) and (1→4)-β-glucans from plants have negative effects because these structures are insoluble and gel forming with the consequence of lower nutrient digestibility in broilers and atrophy of intestinal villi [1,4,5]. On the other hand, several benefits to broilers such as growth performances, immune-modulatory properties and meat quality [6-20] were revealed after feeding supplements with (1→3) and (1→6)-β-glucans from yeast, bacteria or mold. Moreover, the mortality rates of broilers after pathogen challenge test were lower in the group with β-glucans supplementation comparing to the control group fed basal diet without the supplements [10,11,13-15,19,20]. Therefore, β-glucans were considered as immunomodulator supplements in broilers [1] and humans [3]. The research to date has tended to focus on using yeast β-glucans rather than mold (*Ophiocordyceps dipterigena*) which has a similar structure [17]. Interestingly, low molecular weight (5 kDa) β-glucans exhibited the highest function to induce interleukin-8 production comparing to the β-glucans with higher molecular weight [2]. Therefore, the using β-glucans with low molecular weight in broilers should provide better benefits than using β-glucans with high molecular weight.

The objective of this study was to determine the consequences of using different levels and molecular weights of fungal β-glucan as dietary supplementation on productive performances, health, carcass traits and meat quality in broiler chickens.

MATERIALS AND METHODS

Fungal β-glucan preparation

Ophiocordyceps dipterigena BCC 2073 was grown initially on potato dextrose agar (Difco, Becton, Dickinson and Company, Franklin Lakes, NJ, USA) at 25°C for 5 to 7 days. An agar block (1 cm^3) containing the growing culture was cut into small pieces and transferred to 25 mL of potato dextrose broth (Difco, Becton, Dickinson and company, USA) in a 250-mL Erlenmeyer flask. This liquid seed culture was incubated for 5 to 7 days at 25°C on a rotary shaker at a shaking speed of 200 rpm (New Brunswick, Franklin Lakes, NJ, USA). The medium used in a 300-L fermenter consisted of 2.5 g/L yeast extract, 68.98 g/L hydrolyzed cassava starch, 2.62 g/L molasses, 0.5 g/L KH_2PO_4, 0.2 g/L K_2HPO_4, 0.2 g/L $MgSO_4 \cdot 7H_2O$, 0.14 g/L $MnSO_4 \cdot H_2O$, 1 mL/L trace element solution (trace elements consisted of 14.3 g/L $ZnSO_4 \cdot H_2O$, 2.5 g/L $CuSO_4 \cdot 5H_2O$, 0.5 g/L $NiCl_2 \cdot 6H_2O$ and 13.8 g/L $FeSO_4 \cdot H_2O$) and 1 mL/L vitamin solution (Blackmores, Warriewood, NSW, Australia). The culture was agitated at 120 to 180 rpm and aerated at 1 vvm, but pH was not controlled. The cultivation was carried out for 10 days. The culture filtrate was then mixed with four volumes of 95% ethanol, stirred vigorously for 10 to 15 min and stored at 20°C for at least 12 h. β-Glucan was redissolved in distilled water, and any insoluble material was removed by centrifugation at 10,000 *g* for 20 min. The supernatant was then dialyzed (2 kDa molecular weight cut off, Spectrum Laboratories, Rancho Dominguez, CA, USA) against 4 L of distilled water for 24 h and drum dried to achieve high molecular weight β-glucan (943 kDa). γ-Irradiation of the glucan solution was carried out using a cobalt-60 irradiator (Gammacell 220 Excel, MDS Nordion, Ottawa, Canada). The doses applied in this work were 50 kGy at room temperature to obtain the β-glucan size of 8 kDa (low molecular weight). Dosimetry was carried out using 10×30×3 mm of Harwell Red Perspex 4034 (Harwell Dosimeters Ltd., Oxford, UK) calibrated against reference standard dosimeter (Fricke dosimeter) that was traceable to international standard set by the Office of Atoms for Peace, Bangkok, Thailand. The radiated β-glucan solution was then drum dried and stocked for dietary supplement experiments.

Diets, animals, and housing

The basal diets for growing (1 to 21 days old) and finishing period (22 to 42 days old) met the nutrient requirements of Bangkok animal research centre Co., LTD (Bangkok, Thailand). The ingredients and chemical composition of experimental diets are illustrated in Table 1. The β-glucan samples were provided by Asia Star Trade Co., LTD (Bangkok, Thailand) and BIOTEC with purity at 94.5% and 92.5% dry matter (DM) for low and high molecular weight, respectively.

The complete randomized block design was performed in 2×4 factorial arrangement with molecular weight of β-glucan (low or high) and supplemental doses (0, 10, 30, and 60 ppm) as main effects. Two hundred and ten of one-day-old chicks of equal sex from a commercial hatchery (Avian CP, Charoen Pokphand Foods PCL., Bangkok, Thailand) were transported to the Animal Experimental Unit (Faculty of Veterinary Medicine, Kasetsart University, Bangkok, Thailand). The chicks were randomly assigned into 7 experimental groups as follows: i) control group fed the basal diet (C), basal diet with; ii) low dose (10 ppm); iii) medium dose (30 ppm); iv) and high dose (60 ppm) of low molecular weight β-glucan; v) basal diet with low dose (10 ppm); vi) medium dose (30 ppm); vii) and high dose (60 ppm) of high molecular weight β-glucan. Each treatment group contain 5 replicate pens and 6 birds per pen.

All broilers had free access feed and clean water (*ad libitum*), aand were kept in a closed housing system with 18 L:6 D lighting program. The temperature in the room was controlled at 34°C for 5 days and then gradually decreased