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Enhancement of the immunity and body weight gain in broiler by feeding with the brewer's yeast β-glucan degraded by gamma Co-60 radiation

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Abstract: The insoluble *β*-glucan extracted from the cell wall of brewer's yeast was dispersed in deionized water for swelling, then irradiated in order to degrade into water-soluble β-glucan. The results revealed that the water-soluble *β*-glucan contents in the irradiated samples were increased with radiation dose to 25.89, 49.07 and 66.71%; whereas their molecular weight (Mw) decreased to 48.1, 23.0 and 10.8 kDa by gamma irradiation at 100, 200 and 300 kGy, respectively. The supplementation of poultry feed with the radiation degraded *β*-glucan enhanced both non-specific (total white blood cells, lymphocytes, neutrocytes) and specific immune components (anti-Newcastle disease, anti-Gumboro disease virus and anti-infectious bronchitis virus antibodies) in the broilers. In comparison with the control, broiler fed normal poultry foodstuff without β-glucan, the supplementation of radiation degraded *β*-glucan not only increased the survival rate of the testing broiler about 33.3% and their average body weight of about 24.4%, but also reduced the feed conversion rate from 4.8 to 3.1 kg. The *β*-glucan oligosaccharides that having Mw of about 25 kDa produced by gamma irradiation at 200 kGy showed the highest effect on the growth performance and immunomodulatory capability in the immune system of the testing broilers. This product is promising to be applied for production of the safe stimulator of immunity for broiler chickens.

Keywords: *gamma irradiation, brewer's yeast β-glucan, broilers, immunity.*

I. INTRODUCTION

Yeast *β*-glucan has a long chain of approximately 1,500 *β*-1-3-D-glucose units and consist of a backbone of *β*-(1→3)-D-glucopyranosyl units linked with *β*-(1→6) short side chains of varying length and distribution. In food industry, the yeast *β*-glucans have been used for production of salad toppings (dressings), frozen deserts, sauces, yogurts and other milk products, soft doughs and paning doughs, and mixture for cake filling [1,2]. Yeast *β*-glucan is also known to generate antitumor and antimicrobial activities by enhancing the host immune function. Its stimulatory effects on both specific and non-specific immune responses, antimicrobial activity, and tolerance to oral antigens have been demonstrated in mice [3], in fish [4], pig [5,6], broiler [7], shrimp [8]. In addition, the literature has indicated that *β*-glucan can be applied to improve growth performances in pig, fish, shrimp and chicken. However, the activity of these polysaccharides is usually influenced by their molecular weight (Mw) and low Mw β-glucan oligosaccharides displayed the stronger effects on stimulation of immune response, generation of antimicrobial activity and growth

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promotion than high Mw β-glucan polysaccharides [9,10].

Polysaccharides have been reported to be easily degraded by hydrolysis [11,12] or enzymatic treatment [13,14], but gamma irradiation is a useful tool for degradation of alginate, chitosan, *β*-glucan and so on by the cleavage of the glycosidic bonds [10,15-17]. The basic advantages of radiation degradation for polymer include the ability to obtain valuable low molecular weight substances from polymer reproducibly and quantitatively, without the introduction of chemical reagents and special equipments [15]. Moreover, this technology is unique and more environmentally friendly than conventional methods. Although some methods for degradation of *β*-glucan include acid and alkaline hydrolyses, enzymatic digestion, and ultrasound irradiation have been applied [18,19-21], the ionizing radiation was rarely studied in order to degrade *β*-glucan. So far, there have been several reports on stimulation of immune response of *β*-glucan and low molecular weight *β*-glucan oligosaccharides, but the research on radiation degradation of this natural polysaccharide is still limited. Hence, the aim of the present study is to apply the radiation degradation method to prepare the low Mw and water-soluble *β*-glucan for producing the natural growth promoters with immunostimulatory properties for poultry industry.

II. MATERIALS AND METHODS

A. Animals

The chickens (*Gallus gallus domesticus*) with an average body weight about 210 g were supplied by Research Institute of Biotechnology and Environment, Ho Chi Minh City University of Agriculture and Forestry, examined for signs of any disease and all were considered healthy on the basis of a lack of clinically abnormalities.

B. Materials

Brewer's yeast was collected from Saigon-Binhduong Brewery, Saigon-Binhtay Joint stock company. Feedstuffs (Con Co 235) consist of 1.6% crude protein, 6% cellulose, 1.5% calcium, 0.5% salt was purchased from Proconco company. The Kit for analyzing of *β*-glucan (K-YBGL) was supplied by Megazyme International Ireland Ltd.

C. Methods

1. Yeast β-glucan preparation: *β*-glucan was extracted from brewer's yeast cell wall by the method of William et al. [22]. The water-insoluble *β*-glucan was obtained by means of twice extraction of *Saccharomyces carlsbergensis* cells using 3% NaOH at 90^{o}C followed by triple digestions with hydrochloride acid (2.45, 1.75 and 0.94 M) at 90^{o}C. The residue was then washed in turn by diethylether, ethanol and deionized water. After the removal of all soluble components, *β*-glucan was left as the insoluble residue. The final extracted sample contains about 92% *β*-glucan (as analyzed by a K-YBGL Kit, data not shown).

2. Radiation degradation of β-glucan: The obtaining *β*-glucan was suspended, incubated and swollen in deionized water overnight at room temperature, then stirred for 3 h to obtain 10% (w/v) mixtures. The *β*-glucan mixtures were gamma irradiated under Co-60 irradiator for degradation. The processing was carried out at room temperature with radiation dose ranging from 100 to 300 kGy with the same dose rate of 3 kGy/h.

3. Determination of the water-solubility of radiation degraded β-glucan: For determining the difference in water-solubility of the radiation degraded *β*-glucan, the irradiated mixtures were first lyophilized as described by Byun et al. [23]. Two grams of sample powder were put into a 50-ml glass tube with a cap, vortexed with 10 ml deionized water for 20 min, and centrifuged at 3500× g for other 20 min. The supernatant was separated and dried at 100^{o}C for 2 h, and the weight of the dried

products was determined. The water-solubility was calculated as follows:

Water-solubility (%) = 100×(weight of dried supernatant)/(weight of initial β-glucan powder).

4. Mw estimation: Gel permeation chromatography (GPC) was carried out to monitor the changes in the average molecular weight (Mw) of β-glucan by gamma irradiation. GPC was implemented using the Agilent 1100 GPC system (USA) equipped detector RID G1362A and a Bin pump G1312A. Ultrahydrogel columns model 250 and 500 from Waters (USA) (7.8 id × 300 mm) equipped with a Ultrahydrogel guard column from Waters (USA) (6 id × 40 mm) were operated at 40^{o}C and eluted with distilled water at a flow rate of 1.0 ml min^{-1}. The β-glucan sample concentration was 0.1% (w/v) and 20 µl of sample solution was loaded into the GPC system. The column was calibrated using six pullulan standard samples with Mw values of 7.78, 12.2, 23.7, 48, 100 and 380 kDa (Polymer Laboratories, USA).

5. Testing in broiler chickens: The 2-week-old chicks with average body weight about 210 g/head were daily fed with commercial diets with or without 500 ppm β-glucan as control and testing chickens. After 8 weeks, their body weights were measured to calculate average gain and their feed conversion rates (FCR) were also evaluated. The indexes of cellular immunity (total white blood cells (WBC), lymphocytes and neutrophils) and antibodies (anti-Newcastle disease, anti-Gumboro disease and anti-infectious bronchitis virus) in blood of the chickens were analyzed at Veterinery hospital, Ho Chi Minh City University of Agriculture and Forestry. All experiments were repeated three times. Data were statistically analyzed using the ANOVA test. The means were compared using the least significant difference (LSD) at a 5% probability level, and the standard deviations were calculated. Theeffects of β-glucan on the growth and immune response of the chickens were investigated with different molecular weight β-glucan prepared by gamma irradiation at various doses.

III. RESULTS AND DISCUSSIONS

A. Change in water-solubility and Mw of β-glucan by irradiation

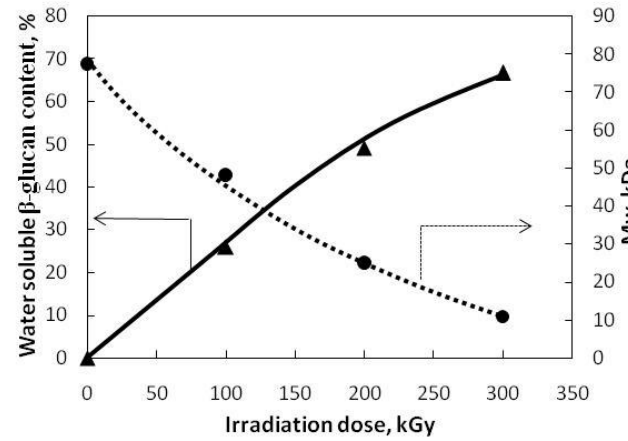

Fig. 1. Change in water soluble content and Mw of β-glucan by gamma irradiation

To prepare the water-soluble β-glucan, several researchers have modified initial β-glucan by carboxylmethylation [18,22]. Degradation to reduce the molecular size or depolymerization are popular ways for preparation of water-soluble β-glucan. In this study, β-glucan extracted from brewer's yeast cell wall is water-insoluble, which were degraded by gamma irradiation, and the radiation effects on the water-solubility and molecular weight of β-glucan were investigated with radiation dose. Fig. 1 indicates that the content of water soluble β-glucan linearly increased with radiation dose, from 25.89% at 100 kGy to 66.71% at 300 kGy. On the contrary, the Mw of water-soluble β-glucan was decreased from 77.4 kDa to 10.8 kDa by the increase of radiation dose up to 300 kGy. These results are in agreement with those reported by Byun et al. [23] and Methacanon et al. [24] that

the gamma irradiation could degrade *β*-glucan in solution, then reduce its Mw and increase the content of water-soluble β-glucan.

B. Effects of radiation degraded *β*-glucan on broilers

The results obtained in Table I indicate that the average body weight of chicken fed by the diet containing 500 ppm non-irradiated *β*-glucan slightly increased, while that value of the chicken fed by irradiated *β*-glucan remarkably increased. These data suggested that *β*-glucan can be used as the growth promoting agent for broiler chickens. In addition, the feed conversion rates (FCR) of the broilers feeding by *β*-glucans irradiated at 200 and 300 kGy are significantly lower than those of control and of broilers supplemented by the initial brewer's yeast *β*-glucan. On the other hand, the results from Table 1 also indicate that the survival rates of boilers fed with low molecular weight *β*-glucan obtained by irradiation at 200 and 300 kGy were about 33.3% higher than that of the control, while the survival rates of the broilers fed with unirradiated *β*-glucan or *β*-glucan irradiated at 100 kGy slightly increased. These results are in good agreement with those obtained from the previous reports [3-5,7,8].

Table I. Effect of irradiation dose for degradation of *β*-glucan on the growth performance and survival of broilers after feeding 8 weeks

Index	Dose, kGy				
	Control*	0	100	200	300
Survival rate, %	61.1^{b**}	66.7^{b}	72.2^{b}	94.4^{a}	94.4^{a}
Feed conversion rate, kg feed/kg body weight	4.8^{a}	3.8^{b}	3.7^{b}	3.1^{c}	3.4^{bc}
The average of total body weight, g/head	1013^{d}	1096^{c}	1152^{b}	1256^{a}	1260^{a}

**, Control: without supplementation of β-glucan; **, Mean values followed by the same letter within a row are not statistically different according to a Duncan's multiple range test at P < 0.05.*

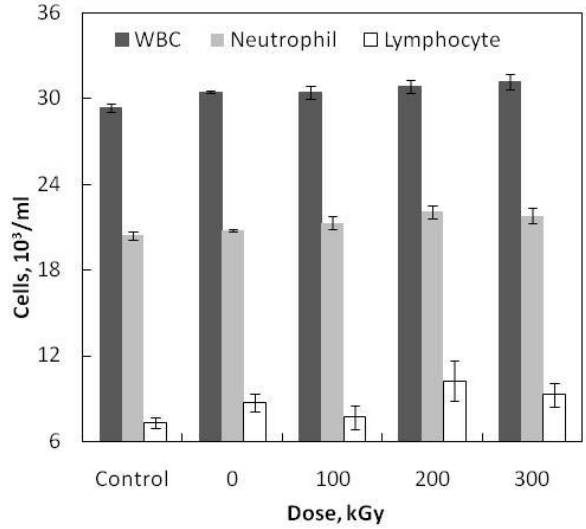

Fig. 2. The cellular immunity indexes in blood of chicken after 8 weeks feeding by commercial diet supplemented with 500 ppm of different *β*-glucan prepared by irradiation at various doses (control: without supplementation with *β*-glucan)

β-glucan has been reported to have positive effects on the immune systems of birds, animals and human include stimulation and modulation. This polymer displayed the stimulation effect on both specific and non-specific immune responses. The results in Fig. 2 indicate that the number of WBCs, lymphocytes and neutrophil cells in blood of the chickens fed with the diet supplemented by β-glucan irradiated at 200-300 kGy are significantly higher than those in blood of the control chicken. These immune cells play a very important role in non-specific immune system for preventing and killing the disease germs when they attacked to broiler chickens. This result is an important reason for explaining why survival rate of the chikens fed by the diet supplemented with β-glucan irradiated at 200-300 kGy was higher than those of control or the chicken fed by the diet supplemented unirradiated β-glucan and 100kGy-irradiated β-glucan. On the other hand, the effect of irradiated β-glucan on specific immune system of chicken was also investigated by analyzing the anti-Gumboro disease, anti-Newcastle disease and anti-infectious bronchitis virus antibody in serum of the broilers. It can be seen from Fig. 3 that the contents of antibodies against the virus causing Gumboro, Newcastle and infectious bronchitis diseases significantly increased by supplementation with irradiated β-glucan for chickens and the highest contents of three checked antibodies are found in serum of the chickens fed with β-glucan irradiated at a dose of 200 kGy.

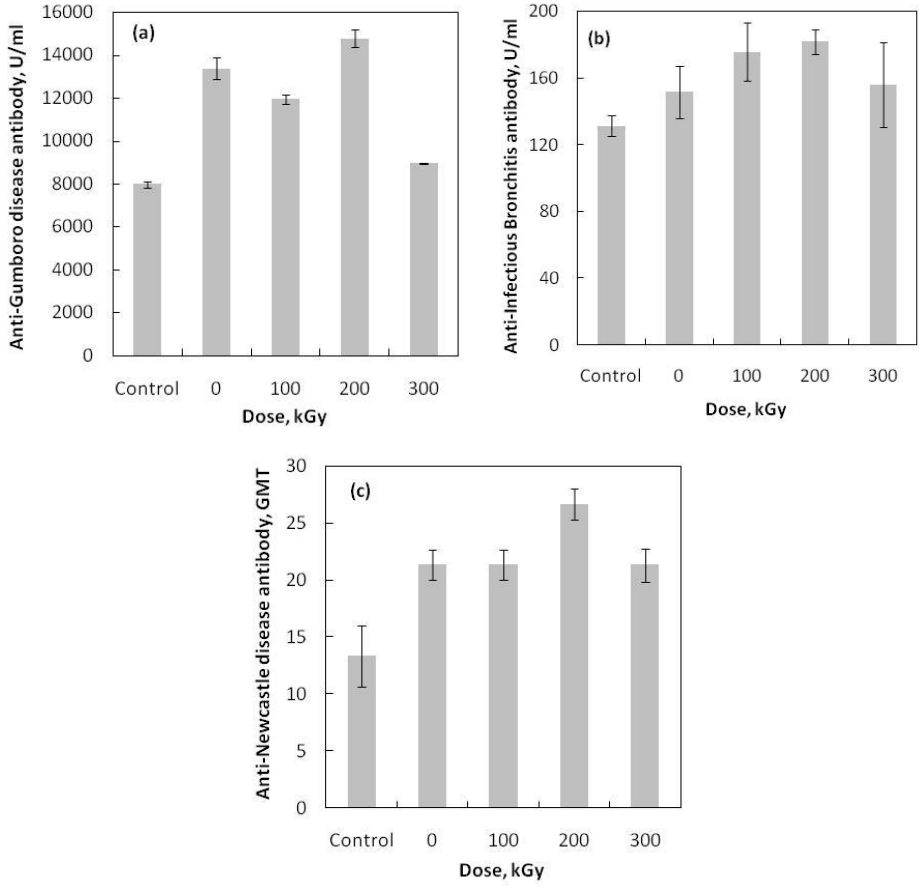

Fig. 3. Effect of the low molecular weight β-glucan prepared by radiation degradation on the content of anti-gumboro disease (a), anti-newcastle disease (b) and inti-infectious bronchitis antibodies (c) in treated broilers

Thus, the obtained results proved that low molecular weight β-glucan of about 25 kDa prepared by radiation degradation at dose of 200 kGy is effective to apply as a supplementation in order to induce the growth performance and immunomodulatory activity for broilers in particular and for poultry in general.

IV. CONCLUSIONS

Gamma irradiation is a useful method for preparation of the water-soluble and low Mw β-glucan. The supplementation of feedstuff with β-glucan having Mw ~ 25 kDa prepared by gamma irradiation at 200 kGy not only improves the average body weight, but also reduces the FCR in chickens. This product also stimulated both specific and non-specifice immune activities in the tested broiler chickens. The radiation degraded β-glucan is promising product to apply as a growth promotion and immunostimulation ingredient for producting value-added quality of feedstuffs for chicken.

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