Hindawi
International Journal of Polymer Science
Volume 2019, Article ID 8901824, 9 pages
https://doi.org/10.1155/2019/8901824

Research Article

Radiation Degradation of β-Glucan Extracted from Brewer's Yeast for Enhancing Growth Promotion and Immunostimulant Activities on Broilers

Nguyen Thanh Long,[1,2] Tran Le Truc Ha,[3] Hoang Nghia Son,[4] and Le Quang Luan[5,6]

[1]*Nha Trang Vaccines and Biological Products Joint Stock Company, Khanh Hoa, Vietnam*
[2]*Graduate University of Science and Technology, Vietnam Academy of Science and Technology, Hanoi, Vietnam*
[3]*Nguyen Tat Thanh University, Ho Chi Minh City, Vietnam*
[4]*Institute of Tropical Biology, Vietnam Academy of Science and Technology, Vietnam*
[5]*Biotechnology Center of Ho Chi Minh City, 2374, Highway 1, Trung My Tay Ward, District 12, Ho Chi Minh City, Vietnam*
[6]*Ho Chi Minh University for Natural Resources and Environment, Ho Chi Minh City, Vietnam*

Correspondence should be addressed to Le Quang Luan; lequangluan@gmail.com

Received 25 September 2018; Revised 25 December 2018; Accepted 3 January 2019; Published 22 January 2019

Academic Editor: Cornelia Vasile

Water-soluble low molecular weight β-glucan (WSLMG) was successfully prepared via γ-irradiation of insoluble β-glucan extracted from brewer's yeast cell walls. The WSLMG content in an irradiated sample increased as the irradiation dose increased. The WSLMGs with a molecular weight (Mw) of 49, 25, and 11 kDa, obtained at correlative doses of 100, 200, and 300 kGy, respectively, were tested using growth promotion and immune stimulant effects in broilers. Supplementation with 500 ppm WSLMGs not only increased the survival rate (33.3%) and average body weight (40%) but also reduced the feed conversion rate (35.4%) in tested broilers. In addition, WSLMGs enhanced both nonspecific and specific immune components in the blood of supplemented broilers. The WSLMG with Mw ~25 kDa showed the highest effect on the growth performance and immunomodulatory capability in the immune systems of the tested broilers. In conclusion, this product demonstrates substantial promise as an immunostimulant and growth-promoting additive for poultry.

1. Introduction

1-3-β-Glucan, a composite of glucose units linked together to form a long polymer chain, is found in the cell walls of fungi, bacteria, and plants [1–3]. Although it is produced from microbes including yeast, mushrooms, bacteria, and from other sources such as seaweeds, cereals, and legumes, β-glucan extracted from yeast cell walls consists of a long chain of approximately 1500 β-1-3-D-glucose units based on the backbone of β-(1→3)-linked β-D-glucopyranosyl units with β-(1→6)-linked side chains of various lengths and distributions [4]. Yeast β-glucans have been widely used in the food industry for production of salad toppings, sauces, yogurts, soft doughs, etc. [5, 6]. The well-known, specific bioactivities of this polymer enhance the host's immune function, leading to antitumour and antimicrobial activities. β-Glucan isolated from yeast has been demonstrated to stimulate responses, tolerance to oral antigens and antimicrobial activity in several types of animals, such as pigs [7, 8], fish [9], mice [10, 11], shrimp [12], and broilers [13]. Furthermore, this biopolymer also displays a stimulatory effect on the growth of pig, fish, shrimp, and chicken [8, 9, 12, 13]. Moreover, low molecular weight (Mw) products from this natural polysaccharide have been shown to have stronger stimulation effects on immune activity in mice [14].

For degradation of polysaccharides, conventional methods have been used, including acidic treatment [15, 16] and enzymatic hydrolysis [17, 18]. These two methods have been demonstrated to decrease molecular size, but a number of disadvantages, including issues related to product

purification, waste treatment, low yield, and high cost, still remain [19, 20]. Gamma irradiation has been proposed as a useful method for degradation of natural polysaccharides, such as cellulose, alginate, and chitosan, via cleaving of glycosidic bonds in the polymer chain [21–23]. The main advantages of radiation degradation for natural polysaccharides include the ability to forego chemicals or special conditions, ease of control, large-scale application, high degradation yield, and low cost [21]. Although this technology is unique and environmentally friendly, low Mw β-glucan products have previously been prepared using chemical hydrolyses or enzymatic digestion [24–27], and research on degradation of β-glucan by ionizing radiation is limited. Hence, the purpose of the present study was to apply the γ-ray irradiation method for degradation of β-glucan extracted from brewer's yeast to prepare WSLMG products. Additionally, the resulting product was tested as a feed additive for broilers with the aim of enhancing growth promotion and immunostimulant effects.

2. Materials and Methods

2.1. Materials. Spent brewer's yeast slurry *(a strain of Saccharomyces carlsbergensis)* with approximately 10.3% dried solid content was obtained from Saigon-Binhduong Brewery, Saigon-Binhtay Bear Joint stock company, Ho Chi Minh City, Vietnam. Chicks (*Gallus gallus domesticus*) with an average body weight of approximately 210 g were supplied by Ho Chi Minh City University of Agriculture and Forestry. The chicks were examined for signs of disease, and all were considered healthy, as they lacked clinical abnormalities. Feedstuffs (Con Co 235) consisted of 1.6% crude protein, 6% cellulose, 1.5% calcium, and 0.5% salt and were purchased from Viet Phap Proconco Joint stock company, Vung Tau, Vietnam. All chemicals and reagents were obtained from Sigma Chemical Co., St. Louis, MO, USA, unless otherwise stated. The kit for analysing β-glucan (K-YBGL) was supplied by Megazyme International Ireland Ltd.

2.2. Preparation of Brewer's Yeast Cell Walls. After collection from the factory, spent brewer's yeast slurry was heated to boil in 5 min and used to kill the yeast cells. The boiled yeast slurry was then centrifuged at 4500 × g for 10 min to collect the yeast cells. After being washed three times with deionized water, the collected yeast cells were kept at 50°C for 24 h for autolysis [28]. The autolysated solution was then centrifuged at 4500 × g for 10 min to retrieve brewer's yeast cell walls with a dried solid yield of approximately 27.9% (w/w). The wet yeast cell walls were kept at 4°C for additional treatments.

2.3. Optimization of Brewer's Yeast β-Glucan Preparation

2.3.1. Effect of NaOH Concentration. Suspensions of 100 g of wet yeast cell walls suspended in 500 ml NaOH with various concentrations (1, 2, 3, and 4%, w/v) were heated at 90°C for 9 h with a continuous stirring condition and then stopped through cooling to room temperature. The reacted suspensions were centrifuged at 4500 × g for 10 min, and the supernatant was removed. The crude solid residue was washed three times with 500 ml of distilled water and recovered by centrifugation (as described above), followed by triple digestions with hydrochloride acid (2.45, 1.75, and 0.94 M) at 90°C [29]. The digested residue was then washed sequentially by diethylether, ethanol, and deionized water. After the removal of all soluble components, β-glucan was left as an insoluble residue.

2.3.2. Effect of Extraction Time, Temperature, and the Sample Ratio. For optimization of the sample to the NaOH solution ratio in the other extraction mixture, the condition was replicated with the aforementioned procedure except the NaOH concentration was 3% with a varied ratio of sample/alkaline solution of 1 : 3, 1 : 5, and 1 : 7 ($w : v$). To study the effect of extraction time, the extraction times were varied at 3, 4, 6, 9, and 12 h. To explore potential temperature effects, the extraction temperatures were varied at 80, 90, and 100°C.

2.4. Chemical Assays. Crude protein of β-glucan samples was measured using the micro-Kjeldahl method, and calculation for the protein content was done by multiplying the total nitrogen by 6.25 (AOAC, 1997). β-Glucan content was analysed via a K-YBGL kit using glucose as a standard, with the procedure performed according to the manufacturer's protocol. All measurements were repeated three times on each sample, and the results are reported as the average based on a dry matter basis.

2.5. Irradiation of the β-Glucan Sample for Degradation. To irradiate the β-glucan sample by gamma rays, 10 g of β-glucan powder was suspended in 100 ml of deionized water and kept overnight at room temperature for swelling and then was stirred for 3 h to prepare a 10% (w/v) suspension mixture. The β-glucan mixture was transferred into a 100 ml duran bottle with a screw cap before exposing to a Co-60 source (Gamma cell GC-5000, BRIT, India) for irradiation. The applied doses for degradation of β-glucan samples were designed in a range of 100 to 300 kGy with a dose rate of 3 kGy/h.

2.6. Water Solubility Determination. The water solubility of irradiated β-glucan was conducted by the method described by Byun et al. [30]. The irradiated samples were first freeze-dried right after irradiation. Following this, 5 g of irradiated samples was put into a 50 ml glass tube with 25 ml of deionized water; the tube was then capped and vortexed for 20 min before being centrifuged at 4500 × g for 20 min. The supernatant was collected and dried at 100°C for 2 h and weighed for examination of the dried water-soluble β-glucan products in the supernatant. Water solubility was calculated as follows: water solubility (%) = 100 × (weight of dried sup ernatant)/(weight of initial irradiated β-glucan powder).

2.7. Mw Determination. In this study, changes in the average molecular weight (Mw) of β-glucan by γ-irradiation were determined via gel permeation chromatography (GPC) using an Agilent 1100 GPC system (USA) equipped with a detector (RID G1362A) and a bin pump (G1312A). Ultrahydrogel Column models 250 and 500 from Waters (USA) (7.8 id × 300 mm), equipped with a guard Ultrahydrogel

Column from Waters (USA) (6 id × 40 mm), were used for monitoring. The β-glucan sample concentration was 0.1% (w/v), and 20 μl of sample solution was loaded into the GPC system. Samples were processed at 40°C and eluted with distilled water at a flow rate of 1.0 ml/min.

2.8. FTIR (Fourier Transform IR) Measurement. Fourier transform infrared (FTIR) spectroscopy of irradiated β-glucans was performed at ambient temperature using a Shimadzu FTIR-8100A spectrophotometer linked with a Shimadzu DR-8030 computer system. Samples were prepared in a KBr pellet formed by well-dried mixtures of 3 mg samples and 100 mg of KBr. All spectra obtained were the results of 128 scans at a spectrophotometer resolution of 4 cm^{-1} in the wavelength region between 4000 and 400 cm^{-1}. For determination of the peak intensity, the spectrum of each sample was made a baseline correction before calculation by an IR solution software.

2.9. Testing in Broiler Chickens. Two-week-old chicks with an average body weight of approximately 210 g/head and in healthy condition were fed daily with commercial diets supplemented with or without β-glucan (as testing or control chickens, respectively). Each treatment was conducted with 30 chicks and 3 replicates. The effects of β-glucan on the growth and immune response of the chickens were investigated by supplementation with 500 ppm unirradiated β-glucan and samples irradiated at doses in the range of 100 to 300 kGy. Tests for concentration effects were performed by feeding the chicks with 250-1000 ppm WSLMG with Mw ~25 kDa. After 8 weeks, the chicks' body weights were determined to calculate the average weight gain and feed conversion rates (FCR). The meat quality indexes of tested chickens such as eviscerated rate, carcass yield, thigh yield, and chest yield were further determined by the veterinary hospital at Ho Chi Minh City University of Agriculture and Forestry. All animal experiments were conducted according to the ethical guidelines issued by Ho Chi Minh City University of Agriculture and Forestry.

2.10. Determination of Cellular Immunity Indexes. The blood of chickens, after the chicks were fed with and without various Mw β-glucans for 8 weeks, was collected and put into heparin-containing tubes for analysis. Cellular immunity indexes, such as total leukocyte, lymphocytes, and neutrophils in blood, were analysed using an automatic haematology analyser, 18-parameter Celltac α (Nihon Kohden, Japan).

2.11. Antibody Assay. Blood samples collected from tested chickens were analysed for specific antibodies related to anti-Newcastle disease virus (NDV), anti-infectious bursal disease virus (IBDV), and anti-infectious bronchitis virus (IBV) at the veterinary hospital at the University of Agriculture and Forestry. The NDV antibody of chickens was studied by determination of the antibody response of chickens using a hemagglutination inhibition test [31]. The IBDV antibody in the serum of birds was determined by using a commercial IBDV-ELISA kit (Synbiotics IBD ProFLOK1, Synbiotics Corporation, USA), which detects anti-IBDV antibodies of the IgG type. The IBV antibody in plasma of the chickens was detected individually by antigen-specific ELISA using an Infectious Bronchitis Antibody Test Kit CK119 (BioChek, USA).

All experiments were carried out in triplicates, and data were analysed using ANOVA (analysis of variance). The means were compared using the least significant difference (LSD) at a 5% probability level, and the standard deviations were calculated. ANOVA and Fisher's protected least significance difference test were used to distinguish differences between means. Significance was assumed at $P < 0.05$.

3. Results and Discussion

3.1. Optimum Condition for Extracting β-Glucan. It can be seen from Figures 1(a), 1(b), and 1(c) that the concentrations of NaOH, temperature, and time of reaction were strongly related to the extraction yield and β-glucan content of the crude product. The increase in the NaOH concentration in the extraction solution, temperature, and time of reaction led to a decrease in crude product yield and protein content, while the content of β-glucan in crude product increased as the aforementioned extraction factors increased. In addition, the results in Figure 1(d) indicated that the reduction in the ratio between wet weight of yeast cell walls and volume of alkali solution led to the decrease in crude product yield and protein content and to an increase in β-glucan content in the crude product. These results align with those reported by Suphantharika et al. [12]. The optimal conditions for extraction of brewer's yeast, *S. carlsbergensis*, can be summarized as follows: NaOH concentration of 3%, temperature of 90°C, reaction time of 9 hr, and a ratio between wet weight of yeast cell walls and NaOH solution of approximately 1 : 5 (w/v). By extracting with these optimum conditions, the extraction yield of β-glucan product was about 16.13%. The final product was water insoluble, and the contents were 91.78% β-glucan and 1.41% protein. This β-glucan product is comparable to the brewer's yeast β-glucan samples previously reported [29, 32].

3.2. Change in Water Solubility and Mw of β-Glucan by Irradiation. The β-glucan product derived from yeast cell walls is water insoluble, creating a substantial barrier to utilization. Thus far, to prepare the water-soluble β-glucan, several researchers have modified its structure by sulfation, phosphorylation, and carboxylmethylation [24, 29, 33]. Additionally, reducing the molecular size of this polymer by degradation is another method for induction of the water-soluble β-glucan. Polysaccharides can be easily degraded in several forms such as in solution or solid states, and the degradation yield of samples in solution has been found to be higher than that in the solid state. Doses up to 500 kGy and sometimes even up to 1000 kGy have been used for degradation of chitosan and alginate in solid powder [34–37], while the dose up to 200 kGy has been applied for irradiation of alginate, chitosan, and carrageenan solutions [38, 39]. In this study, the doses in the range of 100 to 300 kGy were applied for investigating the degradation of water-insoluble β-glucan in suspension

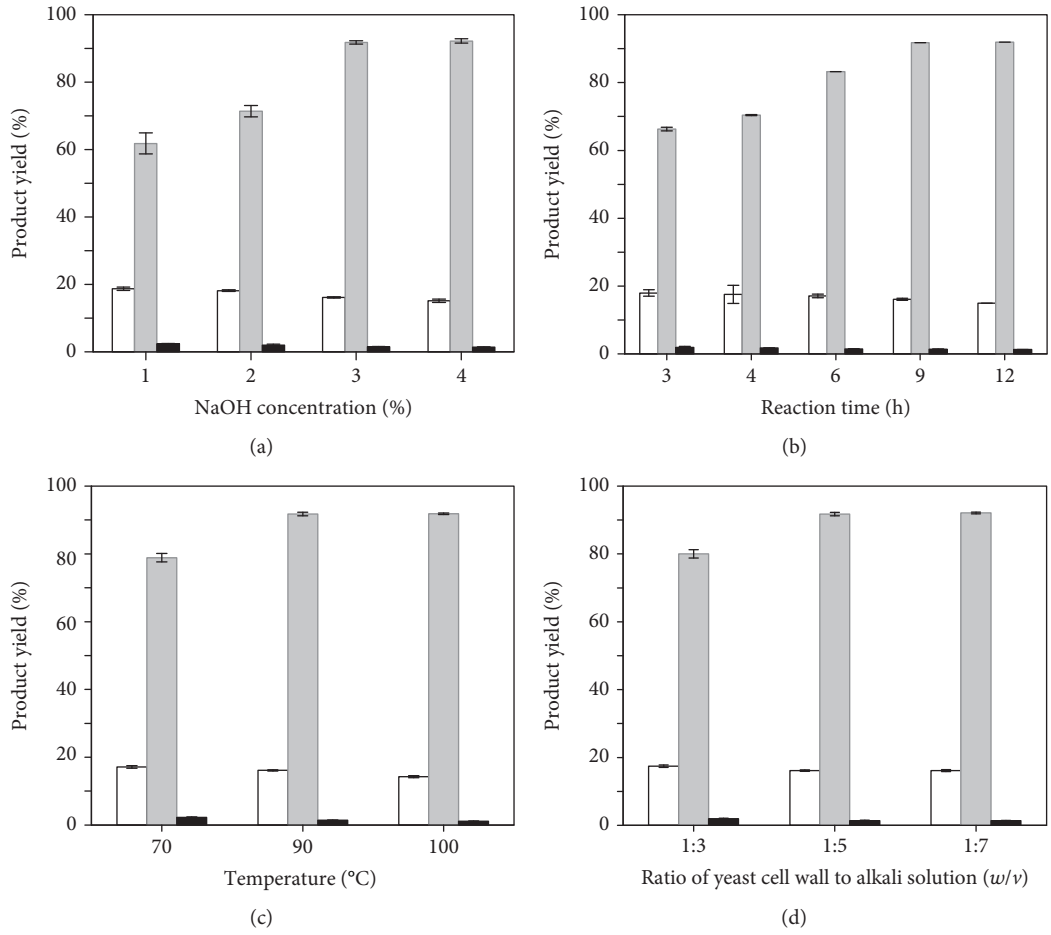

Figure 1: Effect of NaOH content (a), extraction time (b), temperature (c), and alkali solution ratio on the extracted yield of β-glucan; white bar: crude product content, gray bar: β-glucan content in crude product, and dark bar: protein content in product.

condition (swollen state). The results in Figure 2 suggested that the water-soluble content in the sample irradiated at 100 kGy was approximately 25.89%, and this value increased to 66.71% in the sample irradiated at 300 kGy. The results in Figure 2 also showed that the Mw of water-soluble β-glucan from the sample irradiated from 100 to 300 kGy decreased from 49 to 11 kDa, respectively. Thus, it can be seen that the irradiation dose played a key role in reduction of β-glucan Mw. These results are in agreement with those of Byun et al. [30], Methacanon et al. [32], and ours previously [40], which reported that gamma irradiation could cause an increase in water-soluble content and decrease in the Mw of β-glucan in solutions.

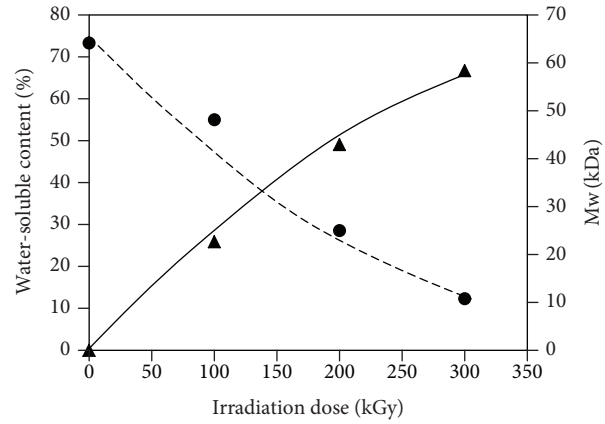

Figure 2: Change in water-soluble content and Mw of β-glucan by gamma irradiation.

3.3. FTIR Analysis. The FTIR spectroscopy technique has been demonstrated as a useful method for monitoring structural changes in biopolymers [41, 42]. The FTIR spectra of irradiated β-glucan are presented in Figure 3. These results are distinguished by the increase in peak intensity at 1731 cm^{-1} assigned to C=O linkages in the end group. It can be seen clearly that only a weak shoulder at 1731 cm^{-1} appears in the spectrum of the native β-glucan, while peaks at this wave number appear in the spectra of irradiated samples, and the peak intensity is increased by the increase of the irradiation dose. Furthermore, the results in Figure 4 show that the ratios between the intensity of the peak, which appears at 1156 cm^{-1} assigned for C-O-C glycosidic linkages, and the intensity of the peak at 1040 cm^{-1} assigned for C-C linkages were proposed to be almost unaffected by irradiation [41, 43] and were reduced by increasing the irradiation dose.

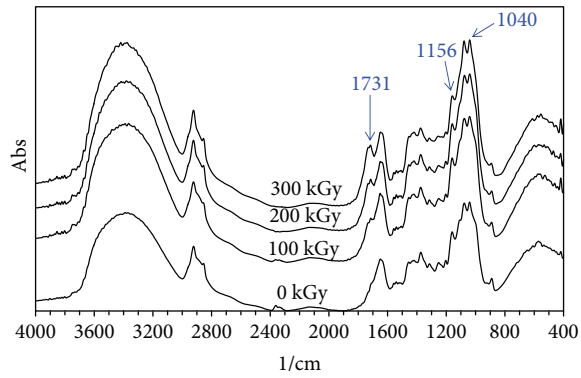

Figure 3: The FTIR spectra of irradiated and unirradiated (1→3)-β-D-glucan samples.

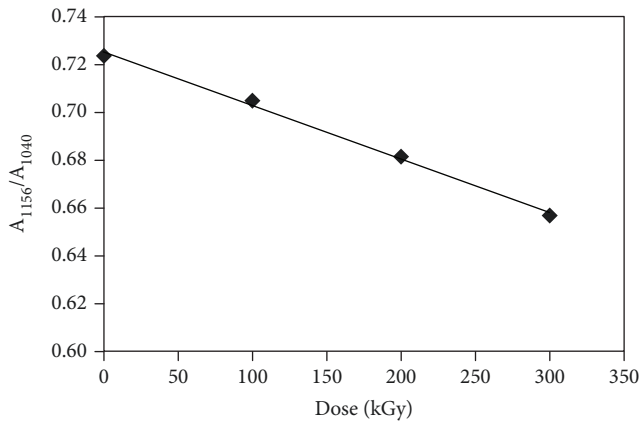

Figure 4: The change of peak intensity by the dose for irradiation of β-D-glucan samples.

This is an evidence that gamma radiation causes scission of the molecular chain of β-glucan at glycosidic linkages. There are several papers reported on degradation of cellulose and its derivatives by γ-rays irradiation proving that the characteristic effect of radiation on natural polysaccharides, whatever the conditions, is degradation due to scission of glycosidic bonds by radiation and not other changes in the structure of polymer molecules. In aqueous solution, the radicals ($^{\cdot}$OH) from irradiated water must be responsible for the degradation of polysaccharides [41, 44], and polysaccharides are typical degradable materials under ionizing radiation through the β-(1–4) or β-(1–6) glycosidic bond cleavage resulting in the reduction of their molecular weights. The main mechanism for degradation of polysaccharides by radiation can be described in the following equation:

$$\mathrm{R-H}+{}^{\cdot}\mathrm{OH} \longrightarrow \mathrm{R}^{\cdot} + \mathrm{H_2O} \longrightarrow \mathrm{F}^{\cdot}_1 + \mathrm{F_2}(\text{scission}). \quad (1)$$

In general, hydroxyl radical reacts with polysaccharide macromolecules (R-H) exceedingly rapid for abstracting a C-bonded H atom and generating $R^{\cdot}$ radicals. These radicals then undergo further reactions to generate lower Mw fragments of the main chain after scission ($F^{\cdot}_1$, F_2) before ending up.

Thus, the differences in form of particular polysaccharides affect not only to their radiation processing but also to degradation efficiency. In the case of irradiation in the powder state, free radicals ($R^{\cdot}$) are formed mainly by disruption of C–H linkages. In the case of irradiation with the presence of water (in solution or swollen condition), the primary free radicals ($^{\cdot}$OH) are formed from irradiated water, and the $^{\cdot}$OH radical is a much more powerful oxidant for disruption of the glycosidic linkages. Therefore, the degradation yield of polysaccharide irradiates in swollen condition is higher than that irradiates in the solid state but lower than that irradiates in solution. The above mechanism can be used for explaining why the applied doses (100-300 kGy) for degradation of the swollen β-glucan sample in this study are higher than that of polysaccharides irradiated in solution (50-200 kGy) [38, 39] but much lower than that of polysaccharides irradiated in the solid form (500-1000 kGy) [34–37].

3.4. Effect of Irradiated β-Glucan on the Growth, Meat Quality, and Immune Index in the Blood of Chickens

3.4.1. Effect of the Irradiation Dose on β-Glucan Degradation. The results in Table 1 indicate that the average body weight of chickens fed the diet containing 500 ppm nonirradiated β-glucan slightly increased, while the value of chickens fed irradiated β-glucan substantially increased compared to the untreated control. In addition, the feed conversion rate (FCR) of the broilers feeding by WSLMGs prepared at the irradiation doses of 200 and 300 kGy was significantly lower than those of the control and supplemented by the nonirradiated brewer's yeast β-glucan. Table 1 also demonstrates that the quality indexes of meat from chickens fed by irradiated β-glucans were better than those of meat from controls and chickens fed with unirradiated β-glucans. These data suggest that irradiated β-glucan can be used as a growth-promoting agent for the broiler chicken. Moreover, among the irradiated β-glucan samples, the sample irradiated at 200 kGy seems to be the most suitable (see Figure 5). The addition of WSLMG prepared by irradiation of 200 kGy (Mw ~25 kDa) increased the average total body weight in approximately 40% of cases, the eviscerated rate in 5.7%, the carcass yield in 9.6%, the thigh yield in 5.6%, and the chest yield in 3% and reduced the feed conversion rate by 35.4% for each kg of chicken weight compared to the untreated control. Additionally, the results from Table 1 indicate that the survival rates of boilers fed with WSLMGs obtained by irradiation at 200 and 300 kGy were approximately 33.3% higher than that of the controls, while the survival rates of the broilers fed with nonirradiated β-glucan or β-glucan irradiated at 100 kGy only slightly increased the survival rates. These results align with those obtained in prior studies [8–10, 12, 13].

β-Glucan has been reported to have positive effects on the immune systems of birds, animals, and humans, including promoting stimulation and modulation. In this study, β-glucan displayed stimulation effects on both specific and nonspecific immune responses. The results in Table 1 also indicate that the number of leukocyte, lymphocyte, and

Table 1: The growth, meat quality, and immune index in blood of broiler chickens after 8 weeks of degraded β-glucan supplementation prepared by various doses.

Index	Dose for irradiation of β-glucan (kGy)				
	Control	0 (unirradiated)	100	200	300
Survival ratio (%)	61.1^{b}	66.7^{b}	72.2^{b}	94.4^{a}	94.4^{a}
The weekly weight gain (g/bird/week)	16.3^{d}	17.8^{c}	18.8^{b}	20.63^{a}	20.7^{a}
The used feed (g/bird/week)	491.1^{a}	475.0^{a}	480.4^{a}	453.3^{b}	479.0^{a}
FCR (kg feed/kg body weight)	4.8^{a}	3.8^{ab}	3.7^{ab}	3.1^{b}	3.4^{ab}
Total body weight (g/bird)	1013^{d}	1096^{c}	1152^{b}	1256^{a}	1260^{a}
The eviscerated rate (%)	70.4^{a}	70.8^{a}	71.4^{a}	76.1^{b}	75^{b}
The carcass yield (%)	50.6^{b}	52.5^{c}	54^{d}	60.2^{a}	58.7^{a}
The thigh yield (%)	20.7^{c}	22.8^{cd}	21.7^{c}	26.3^{b}	25.4^{a}
The chest yield (%)	29.6^{a}	29.7^{a}	31.3^{b}	32.6^{b}	31.8^{b}
Leukocyte (10^3/mm^3)	29.3	30.4	30.4	30.9	31.1
Neutrophil (%)	62.3	61.0	63.0	63.7	63.0
Lymphocyte (%)	25.0	28.7	25.3	31.3	29.7
IBDV antibody (unit)	7980	13,366	11,903	14,770	5613
IBV antibody (unit)	131.5	151.7	175.8	181.9	156.0
NDV antibody (unit)	13.3	21.3	21.3	26.7	21.3

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

(a) (b)

Figure 5: Broiler chicken after 8 weeks supplemented with 500 ppm β-glucan irradiated at 200 kGy (a) and unirradiated β-glucan (b).

neutrophil cells in the blood of the chickens fed with WSLMG-supplemented diets obtained from irradiation doses of 200 and 300 kGy was significantly higher than those in the blood of the control chickens. These immune cells are assumed to play a very important role in nonspecific immune systems for preventing and killing disease germs when they attempt to infect broiler chickens. This important result may help explain why the survival rates of the chickens supplemented with WSLMGs induced by irradiation doses of 200-300 kGy were higher than those of the controls or the chickens fed the diet supplemented with nonirradiated β-glucan and 100 kGy-irradiated β-glucan.

Table 2: The growth, meat quality, and immune index in the blood of chickens after 8 weeks of supplementation by different WSLMG concentrations.

Index	WSLMG concentration (ppm)				
	0	250	500	750	1000
Survival ratio (%)	53.3[b]	73.3[a]	86.7[a]	86.7[a]	86.7[a]
The weekly weight gain (g/bird/week)	16.3[c]	18.1[b]	20.6[a]	20.6[a]	20.6[a]
The used feed (g/bird/week)	446.3[a]	446.6[a]	435.0[a]	444.8[a]	436.6[a]
FCR (kg feed/kg body weight)	4.4[a]	3.6[b]	3.0[c]	3.1[bc]	3.0[c]
Total body weight (g/bird)	1013[c]	1113[b]	1254[a]	1256[a]	1258[a]
The eviscerated rate (%)	69.1[b]	69.1[b]	75.8[a]	76.1[a]	75.0[a]
The carcass yield (%)	51.3[b]	54.1[b]	62.5[a]	60.1[a]	60.1[a]
The thigh yield (%)	21.1[b]	24.3[a]	25.1[a]	25.5[a]	25.5[a]
The chest yield (%)	29.8[b]	29.2[b]	36.1[a]	33.6[b]	34.3[b]
Leukocyte (10^3/mm^3)	30.1	29.7	30.5	29.8	31.0
Neutrophil content (%)	62.7	58.7	67.7	61.3	65.0
Lymphocyte content (%)	25.0	27.0	29.7	25.3	24.3
IBDV antibody (unit)	3031	5627	9629	7079	5991
IBV antibody (unit)	69.27	155.4	212.2	201.1	187.7
NDV antibody (unit)	10.7	10.7	18.7	13.3	13.3

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

The effect of irradiated β-glucan on specific immune systems of chickens was also investigated by analysing the anti-Gumboro disease, anti-Newcastle disease, and anti-infectious bronchitis virus antibody in the serum of the broilers. Table 1 shows that the contents of the antibodies targeting the viruses causing Gumboro, Newcastle, and infectious bronchitis diseases significantly increased after supplementation with irradiated β-glucan, and the highest contents of three checked antibodies are found in the serum of the chickens fed with WSLMG prepared from irradiated doses of 200 kGy.

3.4.2. Effect of WSLMG Concentration. In this experiment, the WSLMG received from the 200 kGy-irradiated β-glucan sample, with Mw estimated at 25 kDa, was used to investigate beneficial concentrations in broilers. The results in Table 2 indicated that the supplementation of WSLMG from 250 to 1000 ppm significantly increased total body weight (from 100 to 245 g/bird, respectively) and survival rate (from 20 to 33.4%, respectively) but reduced the FCR (from 0.8 to 1.4 kg feed/kg body weight, respectively) in broilers after 8 weeks. The meat quality indexes were also found to be significantly increased in chickens supplemented with WSLMG in the range of 250 to 1000 ppm. Additionally, the cellular immunity indexes (i.e., number of WBCs, lymphocytes, and neutrophil ratio) in the blood and specific antibodies (NDV, IBDV, and IBV antibodies) in the serum of the chickens fed with WSLMG were significantly higher than those in the blood and serum of the control chickens, which led to higher survival ratios for supplemented broilers than for the untreated controls. Chae et al. [13] reported that the use of β-glucan up to 200 ppm did not have a significant effect on the growth or immune activity in tested broilers, while supplementation with 400 ppm increased weight gain, feed intake, and lymphocyte subpopulation in broilers as compared to the controls. Our results indicated that supplementation with 250 ppm WSLMG did not have stable effects on the growth, meat quality indexes, or immune activities, but these effects were found to be substantially higher after an increase in supplemented concentration from 500 to 1000 ppm. The 500 ppm of WSLMG as a feed additive seemed to be the most efficient concentration for applications with broiler.

4. Conclusions

Thus, γ-irradiation is a useful method for preparing water-soluble and low Mw β-glucan. The supplementation of feedstuff with WSLMG having Mw ~25 kDa and prepared by γ-irradiation at 200 kGy not only improves the average body weight and meat quality but also reduces the FCR in chickens. The resulting product also stimulated both specific and nonspecific immune activities in the tested broiler chickens. The WSLMG product prepared by γ-irradiation demonstrates promise as a growth promotion and immunostimulation ingredient for producing value-added, quality feedstuffs for chickens.

Data Availability

The data used to support the findings of the water-soluble low molecular weight β-glucans of this study have been deposited in [12, 13, 29, 30, 40]. The data used to support the findings of the FTIR spectra of water-soluble low molecular weight β-glucan in this study have been deposited in [40, 41, 43].

Conflicts of Interest

The authors declare that they have no conflict of interest regarding the publication of this article.

Acknowledgments

This research was supported by the Ministry of Science and Technology of Vietnam (project no. KC.05.16/16-20) and Biotechnology Center of Ho Chi Minh City.

References

[1] A. T. Borchers, J. S. Stern, R. M. Hackman, C. L. Keen, and M. E. Gershwin, "Mushrooms, tumors, and immunity," *Experimental Biology and Medicine*, vol. 221, no. 4, pp. 281–293, 1999.

[2] H. Yamada, "Bioactive carbohydrate polymers, bioactive carbohydrate polymers," *Proceedings of the Phytochemical Society of Europe*, B. S. Paulsen, Ed., Kluwer Academic Publishers, Netherlands, 2000.

[3] G. D. Brown and S. Gordon, "Fungal β-glucans and mammalian immunity," *Immunity*, vol. 19, no. 3, pp. 311–315, 2003.

[4] F. M. Klis, P. Mol, K. Hellingwerf, and S. Brul, "Dynamics of cell wall structure in *Saccharomyces cerevisiae*," *FEMS Microbiology Reviews*, vol. 26, no. 3, pp. 239–256, 2002.

[5] G. Reed and T. W. Nagodawithana, *Yeast-Derived Products and Food and Feed Yeast*, Yeast Technology, Van Nostrand Reinhold, New York, NY, USA, 1991.

[6] V. Zechner-krpan, V. Petravić-tominac, I. Panjkota-krbavčić, S. Grba, and K. Berković, "Potential application of yeast β-glucans in food industry," *Agriculturae Conspectus Scientificus*, vol. 74, pp. 277–282, 2009.

[7] C. R. Stokes, B. G. Miller, M. Bailey, A. D. Wilson, and F. J. Bourne, "The immune response to dietary antigens and its influence on disease susceptibility in farm animals," *Veterinary Immunology and Immunopathology*, vol. 17, no. 1-4, pp. 413–423, 1987.

[8] S. S. Dritz, J. Shi, T. L. Kielian et al., "Influence of dietary β-glucan on growth performance, nonspecific immunity, and resistance to *Streptococcus suis* infection in weanling pigs," *Journal of Animal Science*, vol. 73, no. 11, pp. 3341–3350, 1995.

[9] B. Robertsen, G. RThetaRSTAD, R. Engstad, and J. Raa, "Enhancement of non-specific disease resistance in Atlantic salmon, *Salmo salar* L., by a glucan from *Saccharomyces cerevisiae* cell walls," *Journal of Fish Diseases*, vol. 13, no. 5, pp. 391–400, 1990.

[10] I. Suzuki, H. Tanaka, A. Kinoshita, S. Oikawa, M. Osawa, and T. Yadomae, "Effect of orally administered β-glucan on macrophage function in mice," *International Journal of Immunopharmacology*, vol. 12, no. 6, pp. 675–684, 1990.

[11] J. Liepins, E. Kovačova, K. Shvirksts, M. Grube, A. Rapoport, and G. Kogan, "Drying enhances immunoactivity of spent brewer's yeast cell wall β-D-glucans," *Journal of Biotechnology*, vol. 206, pp. 12–16, 2015.

[12] M. Suphantharika, P. Khunrae, P. Thanardkit, and C. Verduyn, "Preparation of spent brewer's yeast β-glucans with a potential application as an immunostimulant for black tiger shrimp, *Penaeus monodon*," *Bioresource Technology*, vol. 88, no. 1, pp. 55–60, 2003.

[13] B. J. Chae, J. D. Lohakare, W. K. Moon, S. L. Lee, Y. H. Park, and T. W. Hahn, "Effects of supplementation of β-glucan on the growth performance and immunity in broilers," *Research in Veterinary Science*, vol. 80, no. 3, pp. 291–298, 2006.

[14] N. Y. Sung, E. H. Byun, S. K. Kwon et al., "Immune-enhancing activities of low molecular weight β-glucan depolymerized by gamma irradiation," *Radiation Physics and Chemistry*, vol. 78, pp. 433–436, 2009.

[15] M. Hasegawa, A. Isogai, and F. Onabe, "Preparation of low-molecular-weight chitosan using phosphoric acid," *Carbohydrate Polymers*, vol. 20, no. 4, pp. 279–283, 1993.

[16] J. A. Bohn and J. N. BeMiller, "(1→3)-β-d-Glucans as biological response modifiers: a review of structure-functional activity relationships," *Carbohydrate Polymers*, vol. 28, no. 1, pp. 3–14, 1995.

[17] A. V. Ilyina, V. E. Tikhonov, A. I. Albulov, and V. P. Varlamov, "Enzymic preparation of acid-free-water-soluble chitosan," *Process Biochemistry*, vol. 35, no. 6, pp. 563–568, 2000.

[18] T. Shimokawa, S. Yoshida, T. Takeuchi, K. Murata, T. Ishii, and I. Kusakabe, "Preparation of two series of oligo-guluronic acids from sodium alginate by acid hydrolysis and enzymatic degradation," *Bioscience, Biotechnology, and Biochemistry*, vol. 60, no. 9, pp. 1532–1534, 1996.

[19] M.-C. Ralet, M. A. V. Axelos, and J.-F. Thibault, "Gelation properties of extruded lemon cell walls and their water-soluble pectins," *Carbohydrate Research*, vol. 260, no. 2, pp. 271–282, 1994.

[20] Y.-J. Jeon and S.-K. Kim, "Production of chitooligosaccharides using an ultrafiltration membrane reactor and their antibacterial activity," *Carbohydrate Polymers*, vol. 41, no. 2, pp. 133–141, 2000.

[21] A. Charlesby, "Crosslinking and degradation of polymers," *Radiation Physics and Chemistry*, vol. 18, pp. 59–66, 1981.

[22] A. S. Sokhey and M. A. Hanna, "Properties of irradiated starches," *Food Structure*, vol. 12, pp. 397–410, 1993.

[23] M. Cho, B. Y. Kim, and J. H. Rhim, "Degradation of alginate solution and power by gamma-irradiation," *Food Engineering Progress*, vol. 7, pp. 141–145, 2003.

[24] J. Sandula, G. Kogan, M. Kacurakova, and E. Machova, "Microbial (1→3)-β-d-glucans, their preparation, physico-chemical characterization and immunomodulatory activity," *Carbohydrate Polymers*, vol. 38, no. 3, pp. 247–253, 1999.

[25] J. P. Roubroeks, R. Andersson, D. I. Mastromauro, B. E. Christensen, and P. Åman, "Molecular weight, structure and shape of oat (1→3),(1→4)-β-D-glucan fractions obtained by enzymatic degradation with (1→4)-β-D-glucan 4-glucanohydrolase from *Trichodermareesei*," *Carbohydrate Polymers*, vol. 46, no. 3, pp. 275–285, 2001.

[26] H. J. Shin and D. C. Lee, "Study on the process to decrease the molecular weight of beta-(1,6)-branched beta-(1,3)-D-glucans," *The Korean Society for Biotechnology and Bioengineering*, vol. 18, pp. 352–355, 2003.

[27] M. F. Moradali, H. Mostafavi, S. Ghods, and G. A. Hedjaroude, "Immunomodulating and anticancer agents in the realm of macromycetes fungi (macrofungi)," *International Immunopharmacology*, vol. 7, no. 6, pp. 701–724, 2007.

[28] M. Suphantharika, S. Varavinit, and S. Shobsngob, "Determination of optimum conditions for autolyzed yeast extract production," *ASEAN Journal on Science and Technology for Development*, vol. 14, pp. 21–28, 1997.

[29] D. L. Williams, R. B. McNamee, E. L. Jones et al., "A method for the solubilization of a (1→3)-β-D-glucan isolated from *Saccharomyces cerevisiae*," *Carbohydrate Research*, vol. 219, pp. 203–213, 1991.

[30] E. H. Byun, J. H. Kim, N. Y. Sung et al., "Effects of gamma irradiation on the physical and structural properties of β-glucan," *Radiation Physics and Chemistry*, vol. 77, no. 6, pp. 781–786, 2008.

[31] W. Allan and R. Gough, "A standard haemagglutination inhibition test for Newcastle disease. (1). A comparison of macro and micro methods," *Veterinary Record*, vol. 95, no. 6, pp. 120–123, 1974.

[32] P. Methacanon, U. Weerawatsophon, P. Tanjak, P. Rachtawee, and W. Prathumpai, "Interleukin-8 stimulating activity of low molecular weight β-glucan depolymerized by γ-irradiation," *Carbohydrate Polymers*, vol. 86, no. 2, pp. 574–580, 2011.

[33] D. L. Williams, H. A. Pretus, R. B. McNamee, E. L. Jones, H. E. Ensley, and I. W. Browder, "Development of a water-soluble, sulfated (1 → 3)-β-D-glucan biological response modifier derived from *Saccharomyces cerevisiae*," *Carbohydrate Research*, vol. 235, pp. 247–257, 1992.

[34] S. Matsuhashi and T. Kume, "Enhancement of antimicrobial activity of chitosan by irradiation," *Journal of the Science of Food and Agriculture*, vol. 73, no. 2, pp. 237–241, 1997.

[35] N. Q. Hien, N. Nagasawa, L. X. Tham et al., "Growth-promotion of plants with depolymerized alginates by irradiation," *Radiation Physics and Chemistry*, vol. 59, no. 1, pp. 97–101, 2000.

[36] K. Zaman, N. Q. Hien, D. V. Phu et al., "Radiation processing of alginate, chitosan and carrageenan and their application as plant growth promoters," in *The Radiation Chemistry of Polysaccharides*, pp. 197–256, International Atomic Energy Agency, Viena, Austria, 2016.

[37] M. Sen, N. Q. Hien, D. V. Phu et al., "Antimicrobial and antioxidant properties of oligosaccharides," in *The Radiation Chemistry of Polysaccharides*, pp. 257–282, International Atomic Energy Agency, Viena, Austria, 2016.

[38] L. Q. Luan, V. T. T. Ha, N. Nagasawa, T. Kume, F. Yoshii, and T. M. Nakanishi, "Biological effect of irradiated chitosan on plants in vitro," *Biotechnology and Applied Biochemistry*, vol. 41, pp. 49–57, 2005.

[39] L. Relleve, N. Nagasawa, L. Q. Luan et al., "Degradation of carrageenan by radiation," *Polymer Degradation and Stability*, vol. 87, no. 3, pp. 403–410, 2005.

[40] L. Q. Luan and N. H. P. Uyen, "Radiation degradation of (1→3)-β-D-glucan from yeast with a potential application as a plant growth promoter," *International Journal of Biological Macromolecules*, vol. 69, pp. 165–170, 2014.

[41] B. G. Ershov, "Radiation-chemical degradation of cellulose and other polysaccharides," *Russian Chemical Reviews*, vol. 67, no. 4, pp. 315–334, 1998.

[42] A. Ahmad, F. M. Anjum, T. Zahoor, H. Nawaz, and Z. Ahmed, "Extraction and characterization of β-D-glucan from oat for industrial utilization," *International Journal of Biological Macromolecules*, vol. 46, no. 3, pp. 304–309, 2010.

[43] M. Novák, A. Synytsya, O. Gedeon et al., "Yeast β-(1-3),(1-6)-D-glucan films: preparation and characterization of some structural and physical properties," *Carbohydrate Polymers*, vol. 87, no. 4, pp. 2496–2504, 2012.

[44] P. Ulanski and J. Rosiak, "Preliminary studies on radiation-induced changes in chitosan," *International Journal of Radiation Applications and Instrumentation. Part C. Radiation Physics and Chemistry*, vol. 39, no. 1, pp. 53–57, 1992.