Influence of β -glucanase supplementation on growth performance, nutrient digestibility, blood parameters, and meat quality in broilers fed wheat-barley-soybean diet

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Influence of β -glucanase supplementation on growth performance, nutrient digestibility, blood parameters, and meat quality in broilers fed wheat-barley-soybean diet

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Running head: β -glucanase in broiler performance

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Abstract: A total of 600 one-day-old male Ross 308 broiler chickens with an average body weight of 43.00 ± 0.42 g were used in a 35 days growth assay to investigate the supplemental effects of three levels (600, 1200, and 1800 BGU/kg) β -glucanase on growth performance, nutrient digestibility, blood parameters, and meat quality in wheat-barley-soybean meal based broiler diet. During day 19 to 35 and overall period, chickens body weight gain (BWG) and feed conversion ratio (FCR) were linearly significant ($P < 0.05$) improved following the levels of β -glucanase supplementation. The digestibility of crude protein and gross energy were significantly ($P < 0.05$) increased linearly with the levels of β -glucanase supplementation. Significant linear increase ($P < 0.05$) due to increasing β -glucanase supplementation was observed for blood urea nitrogen (BUN) concentration. Increasing β -glucanase supplementation did not influence ($P > 0.05$) the meat quality. In conclusion, broiler diets supplementation with increasing β -glucanase could improve the growth performance, enhance the digestibility, and modulate the concentrations of BUN linearly. Whereas, meat quality was not influenced.

Keywords: β -glucanase, growth performance, nutrient digestibility, meat quality, blood parameters.

Abbreviations: BUN, blood urea nitrogen; BWG, body weight gain; DM, dry matter; FCR, feed conversion ratio; FI, feed intake; MDCP, mono-di-calcium phosphate; N, nitrogen; NSP, non-starch polysaccharides; WHC, water-holding capacity.

Introduction

The application of enzymes for enhancement of nutrient availability in the poultry industry had been reported for a long time, especially after the use of antibiotics has been banned. The water-soluble non-starch polysaccharides (**NSP**) has been demonstrated as an anti-nutritional factor which available abundantly in endosperm cell walls of feedstuff such as wheat, barley, rye and oats fed to poultry (Choct, 2006). It has been clearly investigated that the major cause of growth depression and poor feed conversion in birds fed barley and wheat based diets is mainly due to soluble β -glucans and arabinoxylans (Kiarie et al., 2014). The viscous properties of the soluble NSP, have a negative effect on nutrient absorption leading to poor growth rate and FCR (Mathlouthi et al., 2002a). The benefits of exogenous enzyme supplementation to NSP-rich diets have been widely accepted (Wang et al., 2005; Kiarie et al., 2012; Munir and Maqsood, 2013).

Previously, many studies have reported that dietary supplementation of β -glucanase affect the broiler performance and physico-chemical conditions (Bedford and Cowieson, 2012; Munyaka et al., 2015). Edney et al. (1989) reported that dietary β -glucanase supplement was effective in improving growth and feed conversion of broiler chicks fed on hulled-barley, hulless-barley or oat-groat diets. Ibrahim et al. (2012) proved that dietary supplementation of 0.25g/kg β -glucanase significantly decreased the total feed intake and total feed conversion ratio, increased the weight gain in broilers. As reported by Moharrery et al. (2015), the addition of 0.5g/kg of β -glucanase in broiler diet improved the ether extract digestibility and metabolizable energy corrected for nitrogen.

Furthermore, previous studies also suggested that supplementation of β -glucanase influenced the gut microflora and gastrointestinal morphology in broilers (Brenes et al., 1993; Kiarie et al., 2013). Munyaka et al. (2015) evaluated that enzyme blend with 250 U/kg of β -glucanase changed ileal and cecal microbiota in broiler chickens fed both corn and wheat diets. However, little is known about the effects of dietary supplementation of β -glucanase on blood urea nitrogen (**BUN**) and blood creatinine in broilers.

Therefore, in order to extend previous studies and provide new evidence on blood index, the purpose of the present study was to investigate the effects of β -glucanase supplementation on growth performance, nutrient digestibility, blood parameters, and meat quality in broilers fed wheat-barley-soybean based diet.

Materials and Methods

The experimental protocols describing the management and care of animals were reviewed and approved by the Animal Care and Use Committee of Dankook University, South Korea

Source of β -glucanase

An enzyme preparation (DigeGrain G) used in the present study was provided by Advanced Enzyme Technologies Ltd. (Thane, India). In short, DigeGrain G is a β -glucanase preparation (1800 BGU/g) produced by controlled fermentation of selected strain of *Trichoderma citrinoviride*.

Experimental design, Animals, and Housing

A total of 600 one-day-old male Ross 308 broiler chickens with an average body

weight of 43.00 ± 0.42 g were used in a 5 weeks growth assay. The experiment was conducted into 2 phases consisting of phase 1 (d 1 - 18) and phase 2 (d 19 - 35). Broilers were randomly allotted to 4 dietary treatments containing: CON, wheat-barley-soybean meal based diet; T1, CON + 600 BGU/kg diet DigeGrain G (Advanced Enzyme, India); T2, CON + 1200 BGU/kg diet DigeGrain G; T3, CON + 1800 BGU/kg diet DigeGrain G. All diets were formulated to meet or exceed the NRC (1994) requirement for broiler chickens (Table 1), and all diets were presented in mash form. The enzyme product was weekly mixed properly using a mixer (DDK-801F; Daedong Tech, Korea) according to the manufacturer's protocol. There were 10 replicated pens per treatment with 15 broiler chickens per pen. Broiler chickens were housed in the animal building of Dankook University and practiced an all-in and all-out production system. The room was cleaned every week during the experiment and it was sterilized regularly by using a disinfectant. The temperature of the room was maintained at $33 \pm 1^{\circ}\text{C}$ for the first 3 days, and then gradually decreased by 3°C per week to 24°C until the end of the experiment and the humidity was kept around 60 % through the whole experiment. Artificial light was provided 24 h per day by fluorescent light and was allowed free access to feed and water during the experiment. Each pen was equipped on two feeders in each side and two nipple drinkers.

Sampling and Measurements

Pens were used as the experimental unit in growth performance. The broilers were weighed by pen and feed intake (FI) was recorded on d 1, 18, and 35 to calculate body

weight gain (**BWG**) and feed conversion ratio (**FCR**). Upon completion of the growth assay, 40 chickens (one chicken per pen per treatment) were randomly collected from a wing vein into K₃EDTA vacuum tubes (Becton, Dickinson and Co., Franklin Lakes, NJ). To obtain the serum sample, blood samples were centrifuged at 4,000g for 15 minutes at 4°C, and then stored at –20°C until assay. The BUN concentration and serum creatinine concentration were analyzed using the Abbott Spectrum urea nitrogen test (Series II, Abbot Laboratories, Dallas, TX). Serum glucose concentration was determined using an Astra-8 Analyzer (Beckman Instruments, Inc, Brea, CA 92621).

The chickens used for blood samples were weighed and slaughtered by cervical dislocation in a local commercial slaughterhouse. The breast meat, abdominal fat, gizzard, liver, spleen, and bursa of Fabricius of slaughtered birds were removed by trained personnel. Organ weight and abdominal fat were expressed as a percentage of live BW. Meat color values were measured using a Minolta Chromameter (CR-300, Tokyo, Japan) calibrated with a standard white plate. Meat samples were measured at 3 different locations across the breast and expressed as L* (lightness), a* (redness), and b* (yellowness) values. The pH values of raw breast meat were measured using a digital pH meter (NWKbinaR pH, K-21, Landberg, Germany) after blending 10 g of finely homogenized sample with 90 mL of double-distilled water. The water holding capacity (**WHC**) was determined by the method of Kauffman et al. (1986). Four grams of meat sample was heated to 100°C in a water bath for 30 min. The samples were then cooled with ice and subsequently centrifuged at 5°C at 1,000g for 10 min. Drip loss (%) was

measured on 2 cm × 5 cm × 5 cm thick sections that were removed from broiler breast meat, weighed, and suspended in a zipper bag for 7 d at 4°C. The initial and final weight of each sample was used to calculate the drip loss.

One week before the end of the trial, chromium oxide (Cr₂O₃) was added to the diets at 0.2% level as an indigestible marker to calculate apparent total tract digestibility of dry matter (**DM**), nitrogen (**N**), and gross energy. On the last two days of the experiment, excreta samples were collected from broiler chickens. All feed and excreta samples were pooled by pen and mixed, after which a representative sample was stored in freezer at –20°C until analysis. Before chemical analysis, the excreta samples were thawed and dried for 72 h at 50°C in a forced-air oven (model FC-610, Advantech, Toyo Seisakusho Co. Ltd., Tokyo, Japan), after which they were finely ground to a size that could pass through a 1-mm screen. All feed and excreta samples were analyzed for DM (method 930.15, AOAC, 1995) and N (method 990.03, AOAC, 1995). Chromium was analyzed via UV absorption spectrophotometry (Shimadzu UV-1201, Shimadzu, Kyoto, Japan). Gross energy was determined by using a Compensated Jacket Calorimeter 6100 (Parr Instrument Co., Moline, IL, USA). The content of β-glucan in individual feed ingredients was estimated from the total β-D glucose contents according to the method of Englyst and Cummings (1988) which is used for calculation of β-glucan content of diets.

Calculation and Statistical Analysis

The apparent total tract digestibility was then calculated using the following formula: Digestibility (%) = $[1 - \{(N_f \times C_d) / (N_d \times C_f)\}] \times 100$ where N_f = nutrient

concentration in feces (% DM), Nd = nutrient concentration in diet (% DM), Cd = chromium concentration in diet (% DM), and Cf = chromium concentration in feces (% DM). The pen was used as the experiment unit and all data were analyzed with SAS 2003 (v.9.1, SAS Institute Inc., Cary, NC) using the mixed procedure, with the following statistical model: $Y_{ijk} = \mu + t_i + r_k + e_{ijk}$, where Y_{ijk} is a dependent variable observation, μ is the overall population mean, t_i is the fixed effect of β -glucanase, r_k is the pen as a random diet, and e_{ijk} is the random error associated with the observation ijk . A P-value less than 0.05 was considered significant, and a P-value less than 0.1 was considered as a trend. In addition, orthogonal comparisons were conducted using polynomial regression to measure the linear and quadratic effects of increasing the dietary supplementation of β -glucanase.

Results

Growth performance and nutrient digestibility

The effect of β -glucanase supplementation on growth performance and nutrient digestibility are shown in Table 2 and Table 3, respectively. During phase 1, there were no significant ($P > 0.05$) differences in BWG and FCR among dietary treatments. During phase 2, chickens BWG was significant ($P < 0.001$) linear improvement following the levels of β -glucanase supplementation, and FCR tended ($P = 0.0566$) to decrease. During overall period, BWG and FCR were linearly significant ($P < 0.05$) improved following the increasing levels of β -glucanase supplementation. Whereas, FI was not affected in all phases of experiment. The digestibility of crude protein and gross energy was significant

($P < 0.05$) linear increased with the levels of β -glucanase supplementation.

Blood profiles

Significant linear increase ($P < 0.05$) due to increasing β -glucanase supplementation was observed for BUN concentration. Whereas, the concentration of blood creatinine was tended ($P = 0.0636$) to linear increase (Table 4).

Meat quality

As described in Table 5, increasing β -glucanase supplementation did not influence ($P > 0.05$) the pH, breast muscle color, WHC, cooking loss, drip loss, and relative organ weight.

Discussion

Positive effects on growth performance were observed from phase 2 and the overall period associated with the inclusion of β -glucanase in wheat-barley-soybean meal diets, indicating that β -glucanase exerted beneficial effects on broiler chickens. In agreement with this study, Mathlouthi et al. (2002b) reported exogenous enzyme containing 140000 IU/g activity β -glucanase increased weight gain and FI of broilers fed rye-based diets from 4 to 18 days. Wang et al. (2005) reported that supplementation with 3200 U/g β -glucanase significantly improved ADG and FCR during days 7 to 42 in wheat-based diet. Furthermore, Moharrery et al. (2015) investigated that hulled barley with no treatment had less weight gain and lower efficiency than other cereals. However, these results were not always consistent. Moharrery (2006) reported that weight gain reduced, but no differences in FI or feed conversion in hulled or hulless barley diets with or without β -

glucanase. Rebolé et al. (2010) observed that multi-enzyme contained 8,000 U/g of endo-1,4- β -glucanase and 18,000 U/g of endo-1,3(4)- β -glucanase supplementation to diets had no significant effect on performance parameters. Moreover, Rodríguez et al. (2012) evaluated that commercial product including minimum activity 8000 U/g β -glucanase had no effect on BW gain, FI, and feed conversion in wheat and barley based diet. The mechanism by which dietary soluble NSP have a depressing effect of broiler performance is complex and not fully known. It is assumed that β -glucans and arabinoxylans caused the increase in viscosity of intestinal which depresses nutrient digestibility and might produce changes in the structural morphology of the intestinal wall (Bedford, 1995; Hübener et al., 2002; Parsaie et al., 2007). There were no nutritional limitations in the diet of the control group provided, as it met or exceeded NRC (1994) recommendations for nutrients and energy, thus improved growth performance from β -glucanase addition was not attributed to increased feed consumption but due to improved efficiencies of nutrient use by the bird. The inconsistent finding regarding growth performance in broilers could be due to individual supply of β -glucanase, different feed ingredients or age of broilers.

Juanpere et al. (2005) reported that β -glucanase supplementation to barley diet significantly improved digestibility of DM, lipids and starch. Similar results were found by other researchers (Wang et al., 2005; Rabie et al., 2010). In agreement with their results, broilers fed 1800 BGU/kg of β -glucanase diet had higher digestibility of DM and crude protein in this study. This result conform to the result of the improvement of

growth performance that similar FI among treatments but β -glucanase supplementation treatments improved BWG and FCR. These improvements were probably associated to the reduction of digesta viscosity produced by β -glucanase. The bulk of NSP encrusting the nutrients in barley is composed of β -glucans (Henry, 1985). It could increase the viscosity of barley-based diets, thus, interfere with digestion and absorption of the nutrients. Moreover, it has been reported that with the addition of xylanase and β -glucanase the digestibility of gross energy increased in 21 day old broilers which is in agreement with this study (Cowieson et al., 2010). However, it is in contrast to the study of Yegani and Korver, (2013) in which enzyme treatments had no impact on energy digestibility in broilers. Furthermore, Irish and Balnave (1993) declared soybean meal contains greater content of insoluble NSP as well as represented the major source of amino acid in diet. For instance, Baurhoo et al. (2011) investigated complex enzyme containing 2,000 units/g of endo-1, 3(4) β -glucanase increased DM, crude protein, and essential amino acid digestibility in broilers. In addition, it has reported that 400 U/kg β -glucanase significantly increased the apparent and true metabolizable energy digestibility in different energy levels of diets (Zou et al., 2013). Exogenous β -glucanase can partially hydrolyze the β -glucans and release the enclosed nutrients for the broilers to use. Consequently, broilers can digest the nutrients more easily and achieve better growth performance.

BUN is a medical test that measures the amount of urea nitrogen found in blood. Urea is the primary metabolite derived from dietary protein and tissue protein turnover,

and in mammals, most notably use is nitrogen excretion. The liver produces urea in the urea cycle as a waste product of digestion of protein. It has been recognized that BUN could accurately reflect the state of protein metabolism and balance of amino acids (Borg et al., 1987). Based on BUN concentrations, a study in steers was conducted by Johnson and Preston (1995) to investigate an optimal level of dietary protein for limiting N waste and achieve maximum growth. In addition, Whang and Easter (2000) demonstrated that under fixed conditions, BUN values are correlated with lean gain and feed efficiency in pigs. Moreover, it has been proposed as markers of disease severity with serum creatinine in humans (Koutroumpakis et al., 2016). However, the reports about the effect of β -glucanase on BUN and blood creatinine in broilers is limited. Previously, in a weaning pig trial conducted by Wang et al. (2017), they investigated that the BUN increased in corn-soybean meal diet supplemented with multi-enzyme contained 700 fungal beta-glucanase unit β -glucanase. Furthermore, Yuan et al. (2008) suggested that the BUN concentration was decreased in 180 mg/kg and 360 mg/kg complex enzyme diets compared with corn-soybean meal based broiler diet. Recently, Manoeuvrier et al. (2017) reported that in states of renal hypoperfusion with intact tubular function, BUN is considered to rise out of the proportion to plasma creatinine concentration in humans. In terms of the mechanisms underlying the protein metabolism in broilers, the increasing of BUN may be due to the digestibility of crude protein. Considering the physiology of monogastric animals are similar. Therefore, these findings are not only important for maintaining an appropriate level of β -glucanase in animal health and production, but also

provide insight to improve the health status of humans.

In regard to the meat quality, the analyzed parameters in this study, such as breast muscle color, WHC, cooking loss, drip loss, relative organ weight, and pH value were not affected by supplementation of β -glucanase. Similarly, it was previously shown that the addition of 100 AGL units/kg of β -glucanase in diets did not influence the carcass characteristics including liver, abdominal fat, breast muscle, and leg weight (Lee et al., 2010). Additionally, Mathlouthi et al. (2011) reported that dietary included 70000 BGN/g of β -glucanase activities exogenous enzyme in broiler diets had no effect on yield carcass percentage. Owing to lack of available literature, direct comparisons of response to β -glucanase supplementation in meat quality of broiler is impossible. However, it could be concluded that addition of β -glucanase had no significant deleterious effect on meat quality parameters relevant to consumer acceptability. This conclusion would be worthy to be further investigated.

To summarize, by improving the nutrient absorption of crude protein and gross energy, the supplementation with increasing levels of β -glucanase in broiler diet enhanced the growth performance and modulated the concentrations of BUN linearly. Moreover, meat quality parameters such as meat color, cooking loss, drip loss, pH, WHC, and relative organ weight were not affected which means addition of β -glucanase had no deleterious effect about consumer acceptability. However, further studies are needed to ascertain an appropriate dose of β -glucanase for optimal dietary protein metabolism in broilers.

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Table 1. Composition of control diets (g/kg as fed)

Ingredient, %	Starter (1-18 d)	Finisher (19-35 d)
Barley	20.00	20.00
Soybean meal, 44%	32.68	27.30
Wheat, Hard	36.81	41.19
Tallow	6.28	7.9
MDCP ^c	1.38	1.15
Limestone	1.75	1.48
L-lysine	0.33	0.04
DL-Methionine	0.07	0.05
L-Threonine	—	0.09
Salt	0.20	0.30
Vitamin premix ^a	0.20	0.20
Mineral premix ^b	0.20	0.20
Choline, 50%	0.10	0.10
Total	100.00	100.00
Calculated composition, %		
ME ^c , kcal/kg	3000	3100
Crude protein	22.00	20.00
Calcium	1.00	0.85
Phosphorus	0.72	0.66
Lysine	1.36	1.00
Methionine	0.37	0.33
Met-cys	0.73	0.67
Threonine	0.78	0.79
Tryptophan	0.28	0.26
Beta-glucan	1.22	1.24

^aProvided per kg of complete diet: 11,025 IU vitamin A; 1,103 IU vitamin D₃; 44 IU vitamin E; 4.4 mg vitamin K; 8.3 mg riboflavin; 50 mg niacin; 4 mg thiamine; 29 mg d-pantothenic; 166 mg choline; 33 µg vitamin B₁₂.

^bProvided per kg of complete diet: 12 mg Cu (as CuSO₄•5H₂O); 85 mg Zn (as ZnSO₄); 8 mg Mn (as MnO₂); 0.28 mg I (as KI); 0.15 mg Se (as Na₂SeO₃•5H₂O).

^cMDCP, Mono-di-calcium phosphate; ME, Metabolizable energy.

Table 2. Effect of dietary supplementation of β -glucanase on growth performance in broilers^a

Items	CON	T1	T2	T3	SEM ^b	P value	
						Linear	Quadratic
1-18 d							
BWG ^b , g	748	756	761	764	10	0.2363	0.8166
FI ^b , g	990	993	984	988	4	0.4089	0.8740
FCR ^b	1.324	1.313	1.294	1.292	0.017	0.1550	0.8365
19-35 d							
BWG, g	965	972	979	986	3	<.0001	0.9707
FI, g	1426	1441	1424	1422	15	0.6784	0.5925
FCR	1.478	1.482	1.455	1.442	0.015	0.0566	0.5672
Overall							
BWG, g	1713	1728	1739	1750	11	0.0188	0.8454
FI, g	2416	2434	2408	2409	16	0.5165	0.6073
FCR	1.410	1.408	1.384	1.377	0.008	0.0022	0.7659

Note: ^aAbbreviation: CON, Wheat-barley-soybean meal diets; T1, CON + 600 BGU/kg DigeGrain G; T2, CON + 1200 BGU/kg DigeGrain G; T3, CON + 1800 BGU/kg DigeGrain G.

^bSEM, standard error of means; BWG, body weight gain; FI, feed intake; FCR, feed conversion ratio.

Table 3. Effect of dietary supplementation of β -glucanase on nutrition digestibility in broilers^a

Items, %	CON	T1	T2	T3	SEM ^b	P value	
						Linear	Quadratic
Dry matter	70.08	70.59	70.84	71.04	0.37	0.0742	0.6718
Crude protein	75.45	76.34	76.73	77.22	0.37	0.0025	0.5908
Gross energy	74.64	74.99	75.22	75.49	0.28	0.0404	0.8770
Calcium	59.78	60.80	60.95	61.44	0.80	0.1687	0.7414
Phosphorus	46.03	47.17	47.67	47.97	1.71	0.4177	0.8084

Note: ^aAbbreviation: CON, Wheat-barley-soybean meal Basal diets; T1, CON + 600 BGU/kg DigeGrain G; T2, CON + 1200 BGU/kg DigeGrain G; T3, CON + 1800 BGU/kg DigeGrain G.

^bStandard error of means.

Table 4. Effect of dietary supplementation of β -glucanase on blood profiles in broilers^a

Items, mg/dL	CON	T1	T2	T3	SEM ^b	P value	
						Linear	Quadratic
Blood urea nitrogen	2.3	2.3	2.7	3.0	0.2	0.0021	0.3689
Creatinine	0.16	0.17	0.19	0.19	0.01	0.0636	0.8784

Note: ^aAbbreviation: CON, Wheat-barley-soybean meal Basal diets; T1, CON + 600 BGU/kg DigeGrain G; T2, CON + 1200 BGU/kg DigeGrain G; T3, CON + 1800 BGU/kg DigeGrain G.

^bStandard error of means.

Table 5. Effect of dietary supplementation of β -glucanase on meat quality in broiler^a

Items	CON	T1	T2	T3	SEM ^b	P value	
						Linear	Quadratic
pH value	5.48	5.49	5.44	5.48	0.06	0.7860	0.7681
Breast muscle color							
Lightness (L*)	51.47	51.64	51.55	51.67	0.58	0.8438	0.9703
Redness (a*)	10.24	10.30	10.26	10.37	0.22	0.7326	0.8992
Yellowness (b*)	8.70	8.75	8.74	8.67	0.24	0.9273	0.8033
Water holding capacity, %	57.18	57.17	57.08	57.03	0.63	0.8469	0.9818
Cooking loss, %	18.46	18.28	18.51	18.24	0.33	0.7707	0.8995
Drip loss, %							
day 1	2.53	2.23	2.27	2.19	0.27	0.4294	0.6718
day 3	5.72	5.71	5.67	5.37	0.17	0.1582	0.3927
day 5	8.43	8.11	8.10	7.94	0.23	0.1584	0.7278
day 7	11.59	11.46	11.34	11.25	0.29	0.3922	0.9481
Relative organ weight, %							
Breast muscle	19.36	19.69	19.71	20.14	0.63	0.4063	0.9337
Liver	2.65	2.72	2.71	2.75	0.12	0.6014	0.8923
Bursa of Fabricius	0.13	0.12	0.13	0.13	0.01	0.2847	0.7102
Abdominal fat	1.63	1.61	1.56	1.56	0.09	0.4874	0.8921
Spleen	0.12	0.12	0.12	0.11	0.01	0.6743	0.4175
Gizzard	1.23	1.25	1.24	1.26	0.04	0.6910	0.9900

Note: ^aAbbreviation: CON, Wheat-barley-soybean meal diets; T1, CON + 600 BGU/kg DigeGrain G; T2, CON + 1200 BGU/kg DigeGrain G; T3, CON + 1800 BGU/kg DigeGrain G.

^bStandard error of means.