Effects of hulless barley and exogenous beta-glucanase levels on ileal digesta soluble beta-glucan molecular weight, digestive tract characteristics, and performance of broiler chickens

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ABSTRACT The reduced use of antibiotics in poultry feed has led to the investigation of alternatives to antibiotics, and one such substitution is fermentable carbohydrates. Exogenous β-glucanase (**BGase**) is commonly used in poultry fed barley-based diets to reduce digesta viscosity. The effects of hulless barley (**HB**) and BGase levels on ileal digesta soluble β-glucan molecular weight, digestive tract characteristics, and performance of broiler chickens were determined. A total of 360 day-old broilers were housed in battery cages (4 birds per cage) and fed graded levels of high β-glucan HB (CDC Fibar; 0, 30, and 60% replacing wheat) and BGase (Econase GT 200 P; 0, 0.01, and 0.1%) in a 3 × 3 factorial arrangement. Beta-glucan peak molecular weight in the ileal digesta was lower with 30 and 60 than 0% HB, whereas the peak decreased with increasing BGase. The weight average molecular weight was lower at 0.1 than 0% BGase in wheat diets, whereas in HB diets, it was lower at 0.01 and 0.1 than 0% BGase. The maximum molecular weight was lower with 0.01 and 0.1 than 0% BGase regardless of the HB level. The maximum molecular weight was lower with HB than wheat at 0 or 0.01% BGase. Overall, empty weights and lengths of digestive tract sections increased with increasing HB, but there was no BGase effect. Hulless barley decreased the duodenum and jejunum contents, whereas increasing the gizzard (diets with BGase), ileum, and colon contents. The jejunum and small intestine contents decreased with increasing BGase. Ileal and colon pH increased with increasing HB, but there was no BGase effect. Treatment effects were minor on short-chain fatty acids levels and performance. In conclusion, exogenous BGase depolymerized the ileal digesta soluble β-glucan in broiler chickens in a dose-dependent manner. Overall, feed efficiency was impaired by increasing HB levels. However, HB and BGase did not affect carbohydrate fermentation in the ileum and ceca, although BGase decreased ileal viscosity and improved feed efficiency at the 0.1% dietary level.

Key words: prebiotic, viscosity, oligosaccharide, nonstarch polysaccharide, feed enzyme, fermentation

2021 Poultry Science 100:100967
https://doi.org/10.1016/j.psj.2020.12.064

INTRODUCTION

The continued use of antibiotics in feed has led to the emergence of antibiotic-resistant pathogenic organisms, which is a major concern in the poultry industry (Diarra et al., 2007; Garcia-Migura et al., 2014; Roth et al., 2019). Therefore, the use of in-feed antibiotics has been reduced, and the identification of alternative strategies to antibiotics has become a primary research focus. Comparisons of alternatives to antibiotics on gastrointestinal health, shown by the microbial composition, intestinal morphology and immune response, and production performance of chickens, have been completed in many studies (Gadde et al., 2017; Mehdi et al., 2018; Suresh et al., 2018). Prebiotics are among the most common alternatives to antibiotics that have been studied in poultry (Ducatelle et al., 2015; Pourabedin and Zhao, 2015; Adhikari and Kim, 2017).

Prebiotics are nondigestible nutrient compounds that undergo microbial metabolization in the gastrointestinal tract and result in beneficial physiological effects on the host via different mechanisms (Bindels et al., 2015). However, the most recent definition for prebiotics is

Received August 17, 2020.
Accepted December 20, 2020.

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"a substrate that is selectively utilized by host microorganisms conferring a health benefit," according to the International Scientific Association of Probiotics and Prebiotics (Gibson et al., 2017). The mechanisms whereby prebiotics contribute to host health include changes in gut microbial populations via competitive exclusion (Ofek and Beachey, 1978; Rebolé et al., 2010; Kim et al., 2011) and modification of gastrointestinal morphology (Baurhoo et al., 2009; Chee et al., 2010; Shang et al., 2015) and immune function (Yitbarek et al., 2012; Huang et al., 2015) as a result of increased carbohydrate fermentation (Józefiak et al., 2005; Keerqin et al., 2017). The most common prebiotics studied in the literature are fermentable carbohydrates, and they are, namely, fructo-oligosaccharides, inulin-type fructans, mannan oligosaccharides, and arabinoxylo-/xylo-oligosaccharides (Pourabedin and Zhao, 2015). The dietary addition of wheat arabinoxylo-/xylo-oligosaccharides has been observed to modify gut microbiota, carbohydrate fermentation, and immune function, whereas improving the performance of chickens (Courtin et al., 2008; Eeckhaut et al., 2008; Keerqin et al., 2017).

The present study focused on the effects of low molecular weight β-glucan derived from hulless barley (**HB**) on physiological and performance parameters in chickens. Previous research demonstrates increased production performance of chickens fed barley-based diets with β-glucanase (**BGase**) supplementation (Classen et al., 1988; Edney et al., 1989) due to the reduction of digesta viscosity (Salih et al., 1991; Fuente et al., 1995) and thereby increasing nutrient digestibility (Hesselman and Åman, 1986; Perttilä et al., 2001) and modifying the microbial population in the digestive tract (Choct et al., 1999; Józefiak et al., 2010). Supplementation of barley-based diets with BGase affected carbohydrate fermentation in the ileum and ceca and modulated digestive tract microbial ecology in broiler chickens (Józefiak et al., 2005, 2010), which supports the beneficial effect of BGase supplemented barley β-glucan on microbial fermentation. However, the nature of BGase effects on barley β-glucan that impact carbohydrate fermentation is poorly studied in the literature. Furthermore, BGase sources used in previous research regarding carbohydrate fermentation were not purified and contained a similarly high level of endoxylanase (Józefiak et al., 2005, 2006). Therefore, the effects of BGase on fermentation are confounded by the release of both β-glucan and arabinoxylan. In addition, the prebiotic effect of most of the fermentable carbohydrates, including fructose, mannose, and arabinoxylan, has been tested using the extracted and low molecular weight form (Kim et al., 2011; Pourabedin and Zhao, 2015). Therefore, low molecular weight β-glucan of HB might be a dietary carbohydrate for beneficially affecting the carbohydrate fermentation, gut microbial population, and production performance of chickens. Moreover, the use of a purified form of exogenous BGase helps understand the pure effect of BGase on soluble β-glucan molecular weight and digestive tract characteristics of broiler chickens.

The objective of the study was to evaluate the effects of diet BGase and HB levels on the ileal digesta soluble β-glucan molecular weight distribution, digestive tract characteristics, and performance of the broiler chickens. It was hypothesized that dietary BGase depolymerizes high molecular weight β-glucan, and the resulting low molecular weight β-glucan increases carbohydrate fermentation and beneficially affects the broiler digestive tract morphology and physiology and performance.

MATERIALS AND METHODS

The experimental procedure was approved by the Animal Research Ethics Board of the University of Saskatchewan and conducted according to the Canadian Council on Animal Care guidelines for humane animal use (Canadian Council on Animal Care, 1993, 2009).

Birds and Housing

A total of 360 male (Ross × Ross 308) broiler chickens were obtained from a commercial hatchery and housed in battery cages (51-cm length, 51-cm width, and 46-cm height). The wire mesh floor grid of the cages was 2.54 × 2.54 cm and was covered by a removable 1.27 × 1.27 cm mesh until day 7. Cages were in 2 rows with back to back cages, and each row had 2 levels. The room temperature was adjusted to 32°C at day 0 and was gradually decreased by 2.8°C per week. Day length was 23 h from day 0 to 7 and 18 h from day 8 to 28. A minimum of 25 lux of light intensity was used throughout the trial. Ad libitum feed and water were supplied to the birds throughout the experiment. Each battery cage was equipped with a front-mounted feed trough (51 cm length) and 2 height-adjustable nipple drinkers. Birds were provided with extra feed and water by supplementary chick feeders (plastic, 50 cm long) and ice cube trays (16 cells, L 28.6 cm × W 20 cm × H 3 cm), respectively, until day 5. The bird weight and feed intake (**FI**) were measured on a cage basis. Dietary treatments were randomly assigned to cages, and there were 10 cage replications per treatment and 4 birds per cage.

Experimental Diets

The dietary treatments were designed according to a 3 × 3 factorial arrangement. Hulless barley (CDC Fibar; β-glucan—8.7%) level and BGase (Econase GT 200 P from AB Vista, Wiltshire, UK) level were the 2 factors with HB levels of 0, 30, and 60%, and BGase levels of 0% (0 BU/kg), 0.01% (20,000 BU/kg), and 0.1% (200,000 BU/kg) included in the diets. The diet enzyme level recommended by the manufacturer was 0.005–0.01% (Econase GT 200P, 2019). Hulless barley was added by replacing wheat in each diet, assuming both ingredients have an approximately similar nutrient composition. Hulless barley was ground using a hammermill (VISH M 2014, 140 Parkland, Oak Bluff, MB, Canada) with a screen size of 500 μm. Diets were formulated to meet or exceed Ross 308 broiler nutrition

specifications (Aviagen, 2014) and were fed in crumble form throughout the trial. The ingredients and calculated nutrient levels are presented in Table 1. Titanium oxide was used as an indigestible marker to determine AME_n. The pelleting temperature was maintained at 70°C–75°C to prevent BGase inactivation due to high temperature during feed processing. Beta-glucanase (EC 3.2.1.6) and xylanase activity (EC 3.2.1.8) of the diets were analyzed according to the AB Vista methods of ESC Standard Analytical Method SAM042-01 and SAM038, respectively. The analyzed enzyme activity approximated the calculated values based on enzyme addition, which confirms the correct addition of BGase to the diets, and the enzyme activity was not lost after feed processing (the average BGase activity: 0% BGase, 8,900 U/kg; 0.01% BGase, 23,300 U/kg; 0.1% BGase, 103,967 U/kg). Furthermore, xylanase activity was nondetectable in all the diets (<2,000 U/kg).

Performance Data Collection

The FI and body weight were measured on a cage basis at day 7, 14, 21, and 28 and body weight gain (**BWG**) and feed-to-gain ratio (**F:G**) were calculated. Mortality was recorded daily, and the dead birds were sent to Prairie Diagnostic Services for a detailed necropsy.

Table 1. Ingredients and calculated nutrient levels of experimental diets.

Item	Quantity (%)
Ingredient	
Cereal grain (wheat or hulless barley)[1]	60.00
Wheat (remaining)	5.00
Soybean meal	26.93
Canola oil	4.07
Mono-dicalcium phosphate	1.20
Limestone	1.52
Sodium chloride	0.38
Vitamin-mineral premix[2]	0.50
Choline chloride	0.10
TiO_2	0.30
Nutrient, calculated	
AME (kcal/kg)	3,100
CP	21.24
Crude fat	5.57
Calcium	0.87
Chloride	0.36
Nonphytate phosphorous	0.44
Potassium	0.83
Sodium	0.18
Digestible arginine	1.35
Digestible isoleucine	0.81
Digestible leucine	1.47
Digestible lysine	1.15
Digestible methionine	0.54
Digestible methionine and cysteine	0.87
Digestible threonine	0.77
Digestible tryptophan	0.24
Digestible valine	0.87

[1]Wheat: total dietary fiber (TDF), 15.2; insoluble dietary fiber (IDF), 13.7; soluble dietary fiber (SDF), 1.6; total β-glucan, 0.68/hulless barley: TDF, 29.0; IDF, 19.6; SDF, 9.6; total β-glucan, 8.70 (% DM basis).

[2]Vitamin-mineral premix provided the following per kilogram of the complete diet: vitamin A, 11,000 IU; vitamin D, 2,200 IU; vitamin E, 30 IU; menadione, 2 mg; thiamine, 1.5 mg; riboflavin, 6 mg; pyridoxine, 4 mg; vitamin B_{12}, 0.02 mg; niacin, 60 mg; pantothenic acid, 10 mg; folic acid, 0.6 mg; biotin, 0.15 mg; copper, 10 mg; iron, 80 mg; manganese, 80 mg; iodine, 0.8 mg; zinc, 80 mg; selenium, 0.3 mg; calcium carbonate, 500 mg; ethoxyquin, 0.63 mg; wheat middlings, 3,773 mg.

Sample Collection

Aluminum trays were placed under each cage, and excreta were collected at 12-h intervals for 36 h (3 time points) on a cage basis at day 26 and 27. Feed and feather contaminants were removed, and excreta were collected into plastic bags. Excreta samples were dried using a forced air oven (55°C) and pooled by replication.

All the birds were euthanized at day 28 by intravenous administration of T-61 (Merck Animal Health, Kirkland, Quebec, Canada) into the brachial vein; birds were weighed individually. Two birds per cage were used to collect samples for the analysis of short-chain fatty acids (**SCFA**). Entire ileal and cecal contents were collected into plastic centrifuge tubes and stored at −20°C for the analysis of SCFA. The pH of crop, gizzard, duodenum, jejunum, ileum, ceca, and colon contents was measured in situ using a Beckman Coulter 34 pH meter (Model PHI 34, Beckman Instruments, Fullerton, CA) before collecting contents for the SCFA analysis. Two birds per cage were used to obtain digestive tract size and organ weights. The digestive tract was removed from the bird carcass, and then separated into the crop, proventriculus, gizzard, duodenum, jejunum, ileum, ceca, and colon; organs (the liver, spleen, and pancreas) were removed at the same time. Full weight, empty weight, and the length (when appropriate) of each section of the digestive tract, and organ weights were recorded. The content weight of each section was determined by subtracting the empty weight from the full weight. Empty weight, length, content, and organ weight were divided by individual bird weight to obtain the relative parameters. Ileal contents were collected into plastic snap-cap vials (pooled the contents from 2 birds per cage), and a portion of it was centrifuged at 17,013 × g for 5 min using a Beckman microfuge (model E348720, Beckman instruments, Inc., Palo Alto, CA). The viscosity of the pooled ileal supernatant was measured using a Brookfield cone-plate viscometer (model LVDV-III, Brookfield Engineering Labs, Inc., Stoughton, MA 02072), which was maintained at 40°C (40 rpm; shear rate 300 s^{-1}). The rest of the ileal supernatant derived from centrifugation was stored at −80°C for the analysis of β-glucan molecular weight distribution.

Nutritional Analyses

Experimental diets and ingredients (HB and wheat) were ground to 1 mm (for GE, N, fat, ash, minerals, Ti, and dietary fiber analyses) and 0.5 mm (for β-glucan and total starch analyses) screen-hole sizes using a RETSCH laboratory mill (RETSCH ZM 200, Germany). Beta-glucan was analyzed according to AOAC Method 995.16 (AOAC, 2006), AACC Method 32–23 (AACC International, 2010), and ICC Standard Method No. 168 (ICC, 2011) using a Megazyme analysis kit (Mixed-linkage beta-glucan assay procedure/McCleary

method, Megazyme International Ireland Ltd., Bray Business Park, Bray, Co. Wicklow, Ireland). Insoluble dietary fiber (**IDF**) and soluble dietary fiber (**SDF**) were analyzed using a Megazyme kit (total dietary fiber [**TDF**] assay procedure, Megazyme International Ireland Ltd., Bray Business Park, Bray, Co. Wicklow, Ireland) according to the AOAC method 991.43 (AOAC, 2006) and AACC method 32-07.01 (AACC International, 2010). The TDF was calculated by adding IDF and SDF. The total starch analysis was completed based on the AOAC method 996.11 (AOAC, 2006) and AACC method 76-13.01 (AACC International, 2010) using a Megazyme kit (total starch assay procedure, amyloglucosidase/α-amylase method, Megazyme International Ireland Ltd., Bray Business Park, Bray, Co. Wicklow, Ireland). Nitrogen was analyzed using a LECO nitrogen analyzer (model Leco-FP-528L, LECO Corporation, St. Joseph, MA), and 6.25 was used as the N to CP conversion factor. The fat content was determined by ethyl ether extraction using the Goldfish Extraction Apparatus (Labconco model 35001; Labconco, Kansas, MO) following the AOAC method 920.39 (AOAC, 2006). Ash content was analyzed according to the AOAC method 942.05 (AOAC, 2006) using a muffle oven (model Lindberg/Blue BF51842C, Asheville, NC 28804). Experimental diets were analyzed for the enzyme activity (both BGase and xylanase) according to ESC standard analytical method SAM042-01 and SAM038, respectively, by AB Vista (AB Vista, Wiltshire, UK). Titanium (in the diets and excreta) was determined according to the procedure described by Myers (2014), and moisture was analyzed using the AOAC method 930.15 (AOAC, 2006). Gross energy (in the diets and excreta) was determined using an oxygen bomb calorimeter (model A1435DDEB, Parr Instruments, Moline, IL). Nitrogen-corrected AME was determined using Hill and Anderson (1958) equations.

Beta-Glucan Molecular Weight Distribution

Ileal supernatant was analyzed for β-glucan molecular weight using size-exclusion chromatography, followed by calcofluor postcolumn detection for fluorescent recognition (Boyd et al., 2017). The 2 columns used for HPLC were the Shodex OHpak SB-806M column with OHpak SB-G guard column and a Waters Ultrahydrogel linear column. Tris buffer (0.1 M; pH 8) was used as the mobile phase. The beta-glucan peak molecular weight (**Mp**) and weight average molecular weight (**Mw**) of each sample were determined using a molar mass distribution curve. The molecular weight of the highest β-glucan fraction is referred to as Mp, and the average (based on the weight fraction of each type of molecules) of the molecular weights of all the β-glucan molecules is the definition for Mw. In addition, the maximum molecular weight of the smallest 10% β-glucan molecules (**MW-10%**) was detected using the same distribution curve. The ileal supernatant was boiled for 15 min to inactivate endogenous BGase activity in the sample and then centrifuged for 10 min at 9,000 × *g* using a Beckman microfuge (model E348720, Beckman instruments, INC, Palo Alto, CA) before loading the samples into HPLC.

SCFA Analysis

Short-chain fatty acids were analyzed according to a slightly modified method of Zhao et al. (2006). The internal standard was made using 20 mL of 25% phosphoric acid, 300 μL of isocaproic acid, and deionized water. The standard solution consisted of 300 μL of acetic acid, 200 μL of propionic acid, 100 μL of butyric acid, and 50 μL of isobutyric, isovaleric, valeric, caproic, and lactic acids. Digesta samples were thawed, and phosphoric acid was added at a ratio of 1:1 into it. It was mixed and centrifuged at 12,500 × *g* for 10 min. Then, three aliquots of 1-mL supernatant were taken and mixed with the internal standard at 1:1 ratio. They were filled into microcentrifuge tubes and centrifuged at 12,500 × *g* for 10 min. The supernatant was filtered using a syringe and a 0.45-μm nylon filter and then injected into the gas chromatography column. Thermo Fisher Scientific Gas Chromatograph (model TRACE 1310, Milan, Italy) with Zebron Capillary Gas Chromatography column (ZB-FFAP, length: 30 m; internal diameter: 0.25 mm; film thickness: 0.25 μm, Phenomenex, Torrance, CA) was used for the analysis.

Statistical Analysis

The experiment was a randomized complete block design, and the battery cage level was used as a block to account for potential differences in light intensity and airflow patterns between levels. Each experimental diet had 10 replications (battery cages) with 4 birds per replication, and replications of each treatment were equally distributed in battery cage levels. Data were checked for normality and then analyzed using a two-way analysis of variance (3 × 3 factorial arrangement) of the SAS 9.4 Proc mixed model to determine the main effects of, and interaction between, HB and BGase (SAS 9.4, Carey, NC, 2008). Differences were considered significant when $P \leq 0.05$. The Tukey-Kramer test was used to detect significant differences between means.

RESULTS

Ingredient Nutrient Composition

The content of TDF, IDF, SDF, and total β-glucan in HB was 29.0, 19.6, 9.6, and 8.70%, respectively, and the same parameters were 15.2, 13.7, 1.6, and 0.68%, respectively, in wheat. The content of total starch, CP, fat, and ash in HB was measured as 49.7, 16.2, 2.4, and 2.4% in that order. The same parameters were correspondingly 64.1, 15.0, 1.2, and 1.9% in wheat.

Beta-Glucan Molecular Weight Distribution

An interaction between HB and BGase was found for the β-glucan Mw and MW-10% of the soluble ileal

digesta of broiler chickens (Table 2). In the birds fed 0% HB diets, the Mw was lower with the addition of 0.1% BGase than the 0% BGase. In addition, MW-10% decreased with increasing BGase in wheat-based diets. For the 30% HB treatments, the Mw decreased with the increasing level of BGase, whereas MW-10% was lower with 0.01 and 0.1% BGase than 0% BGase. When considering 60% HB, both Mw and MW-10% were lower with 0.01 and 0.1% BGase levels than with the 0% BGase. No interaction was noticed for Mp of ileal soluble β-glucan. Thus, Mp was higher with 0% than 30 and 60% HB levels, and Mp decreased with the increasing level of BGase in the diets. Figures 1A and 1B indicate ileal soluble β-glucan molecular weight distribution when broilers were fed 60% HB without and with BGase, respectively. The comparison of the proportions of the left side of the blue lines at the point $1e^4$ in the 2 curves demonstrates the low molecular weight β-glucan proportion has been increased with the dietary addition of 0.1% BGase, which follows the MW-10% results in the present study.

AME_n

An interaction between HB and BGase was found for AME_n (Table 3). Nitrogen-corrected AME decreased with the increasing level of HB when the birds were fed diets without BGase. There was no effect of BGase on AME_n at 0 and 30% HB levels. However, AME_n was higher with 0.1% than 0 and 0.01% BGase in the 60% HB treatments.

Viscosity

The interaction between HB and BGase was significant for the viscosity of the soluble ileal content (Table 4). The treatments containing 0.01 and 0.1% BGase resulted in lower ileal viscosity than those containing 0% BGase when the birds were given a wheat-based diet. The ileal viscosity was lower with 0.1 than 0 and 0.01% BGase at 30 and 60% HB levels. However, the HB effect was not significant for ileal viscosity apart from higher viscosity at 30% than 0 and 60% HB at 0.01% BGase.

SCFA and Gastrointestinal pH

There were no HB or BGase effects on ileal and cecal SCFA in broiler chickens except the ileal isobutyric acid (concentration and molar percentage), which was affected by the interaction between main effects, but with no clear trends (Tables 5 and 6). In addition, the ileal valeric acid percentage decreased with increasing BGase in the diets. The concentration of valeric acid also tended to decrease ($P = 0.073$) with increasing BGase. A trend was observed for the interaction of the

Table 2. Effects of hulless barley and β-glucanase on β-glucan molecular weight in the ileal content of broiler chickens aged 28 d.

		Molecular weight (g/mol)		
Hulless barley (%)	β-glucanase (%)	Mp[1]	Mw	MW-10%
0	0	21,536	30,486^{a,b}	9,414^{a}
	0.01	18,276	22,427^{b,c}	5,702^{b}
	0.1	14,324	14,620^{c,d}	2,342^{c}
30	0	19,652	35,863^{a}	5,584^{b}
	0.01	13,255	22,217^{b,c}	3,359^{c}
	0.1	8,952	10,347^{d}	2,025^{c}
60	0	19,799	36,199^{a}	6,099^{b}
	0.01	14,893	16,948^{c,d}	3,407^{c}
	0.1	7,793	8,434^{d}	1,955^{c}
SEM[2]		746.8	1507.8	363.7
Main effects				
Hulless barley (%)				
0		18,045^{a}	22,511	5,819
30		13,953^{b}	22,809	3,656
60		14,162^{b}	20,527	3,820
β-Glucanase (%)				
0		20,329^{a}	34,183	7,032
0.01		15,475^{b}	20,531	4,156
0.1		10,356^{c}	11,134	2,107
ANOVA *P*-value				
Hulless barley		<0.001	0.363	<0.001
β-Glucanase		<0.001	<0.001	<0.001
Hulless barley × β-glucanase		0.206	0.035	0.001

[a–d]Means within a main effect or interaction not sharing a common superscript are significantly different ($P \leq 0.05$).

[1]Mp = peak molecular weight; Mw = weight average molecular weight; MW-10% = maximum molecular weight for the smallest 10% molecules.

[2]SEM = pooled SEM (n = 6 cages per treatment).

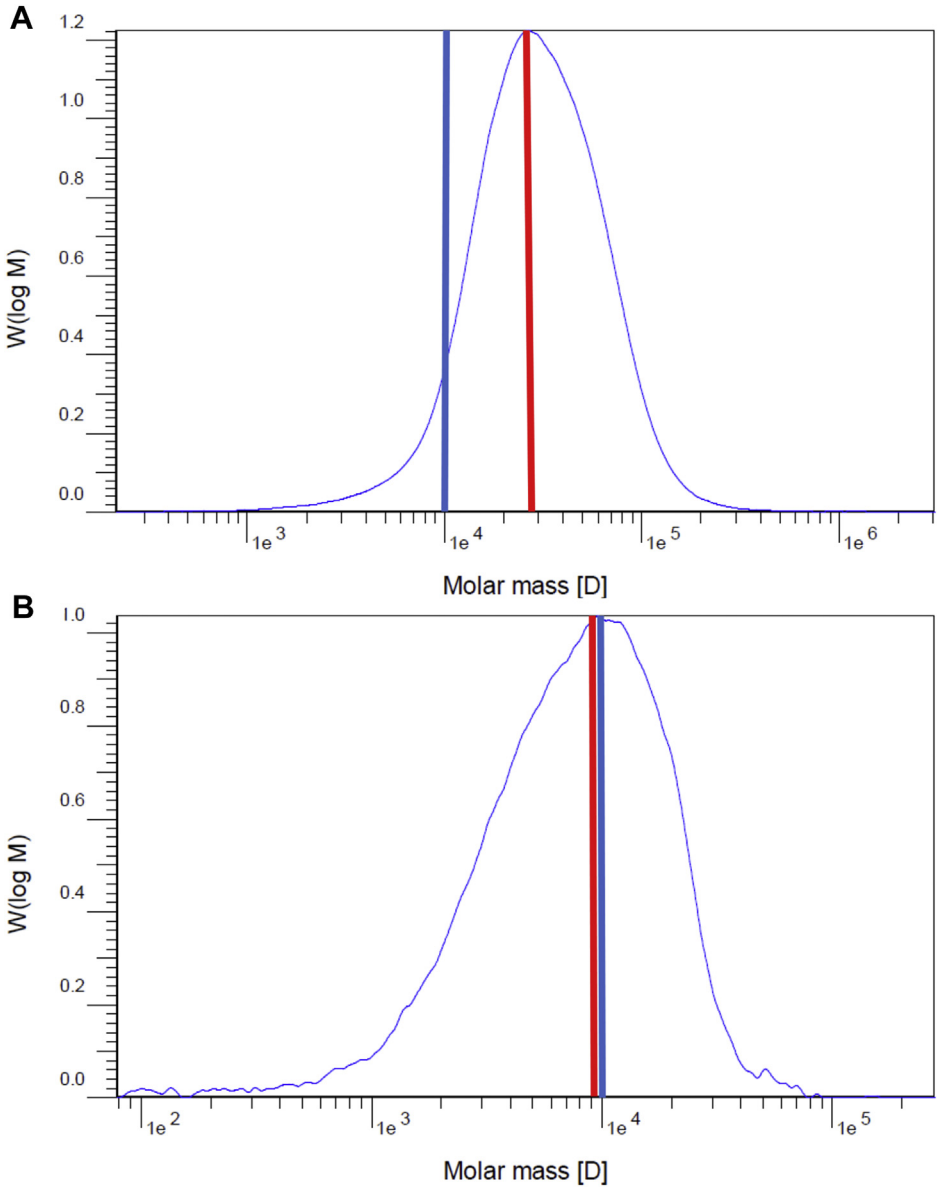

Figure 1. Beta-glucan molecular weight distribution in soluble ileal digesta from broilers fed 60% hulless barley diets. Blue lines denote point $1e^4$ on the x-axis, and red lines indicate the Mp of the distribution curve. (A) 0% β-glucanase; (B) 0.1% β-glucanase. Abbreviation: Mp, peak molecular weight.

main effects on cecal acetic acid percentage ($P = 0.071$), but differences were minor. Ileal and colon pH were higher with 30 and 60% than 0% HB. Furthermore, a trend was noticed for the main effect interaction for the gizzard and cecal pH ($P = 0.057$–0.070) (Table 7).

Digestive Tract Morphology

Interactions between HB and BGase were observed for the empty weights of the crop, gizzard, duodenum, small intestine, and colon (Table 8). The crop weight increased with the increasing level of HB in the broilers fed diets without BGase. The beta-glucanase effect was not significant at 0 and 30% HB treatments. However, the crop weight was higher with 0% than 0.01% BGase when the birds were fed 60% HB. The gizzard weight was higher with 0.01% than 0% BGase for the birds fed 60% HB, although BGase did not affect the gizzard weight at 0 and 30% HB levels. Less clear interactions were found for the empty weights of the duodenum and small intestine. The colon weight was higher, with 0.01% than 0.1% BGase in the birds fed 60% BGase diets; however, no BGase effect was noted at 0 and 30% HB levels. The empty jejunum weight was higher at 0% than 30% HB, and the empty weight of the ileum was higher at 60% than 0 and 30% HB. Interactions between HB and BGase were found for the lengths of the duodenum, small intestine, and colon. The highest duodenum length was found for the birds fed 60% HB diets with 0.01% BGase, whereas the 0% HB treatments had the lowest values; intermediate lengths were noted for the remaining treatments. The small intestine length was higher with 0% than 0.01% BGase when the birds were given wheat-based diets, although the BGase effect was not significant at 30 and 60% HB levels. The colon length was longer at 60 than 0 and 30% HB when the birds were fed 0.01% BGase added diets. In addition, the lengths of the jejunum, ileum, and ceca were higher

Table 3. Effects of hulless barley and β-glucanase on AME_n of broiler chickens aged 28 d.

Hulless barley (%)	β-Glucanase (%)	AME_n (kcal/kg, 90% DM basis)
0	0	3,001[a]
	0.01	2,938[a,b]
	0.1	2,964[a,b]
30	0	2,898[b]
	0.01	2,938[a,b]
	0.1	2,954[a,b]
60	0	2,724[c]
	0.01	2,704[c]
	0.1	2,888[b]
SEM[1]		12.4
Main effects		
Hulless barley (%)		
0		2,967
30		2,930
60		2,772
β-Glucanase (%)		
0		2,874
0.01		2,860
0.1		2,935
ANOVA *P*-value		
Hulless barley		<0.001
β-Glucanase		<0.001
Hulless barley × β-glucanase		<0.001

[a–d]Means within a main effect or interaction not sharing a common superscript are significantly different ($P \leq 0.05$).
[1]SEM = pooled SEM (n = 10 cages per treatment).

at 60% than 0% HB and were higher or equal than 30% HB. There was an interaction between BGase and HB levels on the gizzard content (Table 9). The gizzard content increased with the increasing level of HB in the diets containing 0.01% BGase. Furthermore, the gizzard content was lower with 0% than 0.01% BGase for the 60% HB diets. The duodenum and jejunum content weights were lower at 30 and 60% than 0% HB, and the jejunum content decreased with the increasing level of BGase. The ileum and colon content weights were higher at 60% than 0 and 30% HB. The small intestine content was higher at 60% than 30% HB, whereas the content decreased with the increasing level of BGase. The pancreas weight increased with the increasing level of HB, whereas the pancreas weight decreased at the level of 0.1 compared with 0.01% BGase.

Table 4. Effects of hulless barley and β-glucanase on ileal viscosity of broiler chickens aged 28 d.

Hulless barley (%)	β-Glucanase (%)	Viscosity (cP)
0	0	5.39[a]
	0.01	4.19[b,c,d]
	0.1	3.78[c,d]
30	0	5.00[a,b]
	0.01	5.31[a]
	0.1	3.53[d]
60	0	4.75[a,b,c]
	0.01	3.90[c,d]
	0.1	3.33[d]
SEM[1]		0.114
Main effects		
Hulless barley (%)		
0		4.45
30		4.61
60		3.99
β-Glucanase (%)		
0		5.04
0.01		4.47
0.1		3.54
ANOVA *P*-value		
Hulless barley		0.006
β-Glucanase		<0.001
Hulless barley × β-glucanase		0.010

[a–d]Means within a main effect or interaction not sharing a common superscript are significantly different ($P \leq 0.05$).
[1]SEM = pooled SEM (n = 10 cages per treatment).

Performance Parameters

There were significant effects of HB and BGase on performance parameters at different periods of the experiment (Table 10). Interactions between HB and BGase were found for broiler BWG for all the periods except day 21–28. Overall, the BWG decreased with increasing HB when considering all the periods. For 0 and 30% HB levels, BGase did not affect the BWG in any of the periods. In 60% HB treatments, the BWG was lower with 0.01 and 0.1% than 0% BGase in the birds aged 0–7 d. However, after day 7, there was no BGase effect on BWG at 60% HB except at the period of day 0–28, as the BWG was lower with 0.01% than 0% BGase.

The FI at day 0–7 was lower at 0.1% than 0 and 0.01% BGase in the diets. The interaction between HB and BGase was found for the FI at day 14–21 and the total period. However, BGase did not affect the FI regardless of the HB level except for day 0–28, when it was lower at 0.01% than 0% BGase when the birds were given 60% HB-based diets.

The interaction between the main effects was significant for the F:G at day 0–7 and 7–14. Overall, the F:G increased with the increasing level of HB at day 0–7 and 7–14. The BGase effect was not noticed at 0 and 30% HB. However, the F:G was higher with 0.01 and 0.1% than 0% BGase when the birds were fed 60% HB-based diets at day 0–7. In addition, the F:G was higher at 0.01% than 0.1% BGase in the birds fed 60% HB at day 7–14. The interaction was not found for the F:G at day 14–21 and 21–28. However, the F:G was higher with 60% than 0 and 30% HB at both periods. The F:G increased with increasing HB when considering the total study period. In contrast, the F:G was lower at 0.1% than 0 and 0.01% BGase levels.

The overall mortality of the study was 3.1% and was not affected by HB or BGase.

DISCUSSION

Beta-glucan molecular weight distribution in the soluble ileal digesta was assessed to determine the effect of exogenous BGase on depolymerizing high molecular weight soluble β-glucan in broiler chickens. The ileal Mw did not significantly change with the increasing level of HB in the diets, although the interaction between the

Table 5. Effects of hulless barley and β-glucanase on ileal short-chain fatty acids of broiler chickens aged 28 d.

		SCFA μmol/g of wet ileal content									Molar percentage of total SCFA							
HB[1] (%)	BGase (%)	Total	Ace	Pro	Buty	Isob	Val	Isov	Cap	Lac	Ace	Pro	Buty	Isob	Val	Isov	Cap	Lac
0	0	175.3	65.2	24.4	11.2	2.3^{a,b}	3.4	3.1	1.5	63.8	37.2	13.9	6.4	1.3^{a,b}	1.9	1.7	0.8	36.3
	0.01	172.9	64.9	24.1	11.2	2.2^{a,b}	2.9	3.0	1.4	62.9	37.5	13.9	6.4	1.2^{a,b}	1.7	1.7	0.8	36.3
	0.1	164.5	62.1	23.2	10.3	1.8^{a,b}	2.2	2.4	1.0	61.0	37.9	14.1	6.3	1.0^{a,b}	1.3	1.4	0.6	37.0
30	0	160.3	61.9	22.1	10.0	1.9^{a,b}	2.6	2.5	1.0	58.0	38.7	13.4	6.4	1.2^{a,b}	1.6	1.5	0.6	36.2
	0.01	167.5	63.2	22.6	10.7	2.8^{a,b}	2.6	2.5	1.1	61.8	37.9	13.3	6.3	1.5^{a,b}	1.5	1.5	0.6	37.0
	0.1	171.1	65.4	23.3	10.9	1.6^{a,b}	2.4	2.7	1.2	63.2	38.2	13.4	6.4	0.9^{a,b}	1.4	1.5	0.7	37.2
60	0	165.9	61.8	22.3	10.5	2.7^{a,b}	3.3	2.9	1.3	60.6	37.5	13.1	6.4	1.6^{a,b}	1.9	1.7	0.7	36.6
	0.01	182.5	70.4	25.8	11.3	1.1^{b}	2.8	2.8	1.3	66.6	38.5	14.1	6.2	0.7^{b}	1.5	1.5	0.7	36.4
	0.1	157.2	59.1	20.8	10.3	2.9^{a}	2.6	2.2	1.0	58.0	37.6	13.3	6.5	1.8^{a}	1.6	1.4	0.6	36.9
SEM[2]		3.15	1.17	0.51	0.24	0.13	0.12	0.12	0.05	1.16	0.14	0.16	0.08	0.07	0.06	0.06	0.02	0.15
Main effects																		
HB (%)																		
0		170.9	64.1	23.9	10.9	2.1	2.9	2.8	1.3	62.6	37.5	14.0	6.4	1.2	1.6	1.6	0.7	36.5
30		166.3	63.5	22.7	10.5	2.1	2.5	2.6	1.1	61.0	38.3	13.3	6.4	1.2	1.5	1.5	0.6	36.8
60		168.5	63.8	23.0	10.7	2.3	2.9	2.7	1.2	61.7	37.9	13.5	6.4	1.4	1.7	1.5	0.7	36.6
BGase (%)																		
0		167.2	63.0	22.9	10.6	2.3	3.1	2.8	1.3	60.8	37.8	13.5	6.4	1.4	1.8^{a}	1.7	0.7	36.4
0.01		174.3	66.2	24.2	11.0	2.0	2.8	2.8	1.3	63.7	38.0	13.8	6.3	1.1	1.6^{a,b}	1.6	0.7	36.6
0.1		164.2	62.2	22.5	10.5	2.1	2.4	2.4	1.1	60.7	37.9	13.6	6.4	1.2	1.4^{b}	1.4	0.6	37.0
ANOVA *P*-value																		
HB		0.823	0.980	0.555	0.796	0.858	0.406	0.630	0.431	0.837	0.146	0.240	0.978	0.546	0.471	0.814	0.510	0.793
BGase		0.375	0.318	0.346	0.625	0.615	0.073	0.331	0.174	0.438	0.862	0.795	0.921	0.404	0.050	0.350	0.137	0.249
HB × BGase		0.462	0.301	0.349	0.759	0.003	0.702	0.700	0.281	0.456	0.241	0.786	0.956	0.001	0.599	0.870	0.423	0.887

[a-d]Means within a main effect or interaction not sharing a common superscript are significantly different ($P \leq 0.05$).

[1]HB, hulless barley; Bgase, β-glucanase; SCFA, short-chain fatty acids; Ace, acetic acid; Pro, propionic acid; Buty, butyric acid; Isob, isobutyric acid; Val, valeric acid; Isov, isovaleric acid; Cap, caproic acid; Lac, lactic acid.

[2]SEM = pooled standard error of mean (n = 20 birds per treatment).

Table 6. Effects of hulless barley and β-glucanase on cecal short-chain fatty acids of broiler chickens aged 28 d.

		SCFA μmol/g of wet cecal content								Molar percentage of total SCFA						
HB[1] (%)	BGase (%)	Total	Ace	Pro	Buty	Isob	Val	Isov	Cap	Ace	Pro	Buty	Isob	Val	Isov	Cap
0	0	283.9	168.8	58.6	26.6	8.7	8.6	8.6	3.7	59.3	20.6	9.5	3.0	3.0	3.0	1.3
	0.01	285.0	167.5	59.4	27.8	8.8	8.7	8.7	3.7	58.8	20.7	9.7	3.0	3.0	3.0	1.3
	0.1	281.0	166.5	58.1	26.7	8.6	8.5	8.6	3.7	59.2	20.6	9.5	3.0	3.0	3.0	1.3
30	0	269.4	159.1	55.5	25.4	9.3	8.1	8.2	3.5	59.0	20.5	9.5	3.5	3.0	3.0	1.3
	0.01	248.1	148.7	48.9	24.5	7.6	7.4	7.5	3.2	60.0	19.5	9.9	3.0	3.0	3.0	1.3
	0.1	277.9	164.0	57.6	27.3	8.5	8.4	8.1	3.6	58.9	20.7	9.8	3.0	3.0	2.9	1.3
60	0	284.1	166.7	58.3	28.0	9.9	8.6	8.6	3.7	58.7	20.5	9.8	3.5	3.0	3.0	1.3
	0.01	282.9	166.6	58.5	27.9	8.7	8.6	8.6	3.7	58.9	20.6	9.8	3.0	3.0	3.0	1.3
	0.1	274.4	162.0	56.5	27.1	8.4	8.3	8.3	3.5	59.0	20.6	9.9	3.0	3.0	3.0	1.3
SEM[2]		4.70	2.76	1.05	0.45	0.21	0.15	0.15	0.06	0.10	0.10	0.05	0.06	0.01	0.01	0.003
Main effects																
HB (%)																
0		283.3	167.6	58.7	27.0	8.7	8.6	8.6	3.7	59.1	20.6	9.6	3.0	3.0	3.0	1.3
30		272.0	157.3	54.0	25.8	8.5	8.0	7.9	3.4	59.3	20.2	9.7	3.2	3.0	3.0	1.3
60		277.8	165.1	57.8	27.7	9.0	8.5	8.5	3.6	58.8	20.5	9.8	3.2	3.0	3.0	1.3
BGase (%)																
0		279.1	164.9	57.5	26.7	9.3	8.4	8.5	3.6	59.0	20.5	9.6	3.3	3.0	3.0	1.3
0.01		272.0	161.0	55.6	26.7	8.4	8.2	8.3	3.5	59.2	20.3	9.8	3.0	3.0	3.0	1.3
0.1		277.8	164.2	57.4	27.0	8.5	8.4	8.3	3.6	59.1	20.6	9.7	3.0	3.0	3.0	1.3
ANOVA *P*-value																
HB		0.227	0.273	0.140	0.202	0.583	0.233	0.149	0.239	0.168	0.234	0.133	0.594	0.862	0.473	0.805
BGase		0.775	0.801	0.672	0.945	0.165	0.805	0.866	0.801	0.546	0.376	0.270	0.135	0.632	0.578	0.632
HB × BGase		0.683	0.795	0.432	0.589	0.580	0.647	0.851	0.643	0.071	0.146	0.710	0.699	0.968	0.733	0.963

[a-d]Means within a main effect or interaction not sharing a common superscript are significantly different ($P \leq 0.05$).
[1]HB, hulless barley; Bgase, β-glucanase; SCFA, short-chain fatty acids; Ace, acetic acid; Pro, propionic acid; Buty, butyric acid; Isob, isobutyric acid; Val, valeric acid; Isov, Isovaleric acid; Cap, caproic acid; Lac, lactic acid.
[2]SEM = pooled standard error of mean (n = 20 birds per treatment).

Table 7. Effects of hulless barley and β-glucanase on the gastrointestinal pH of broiler chickens aged 28 d.

Hulless barley (%)	BGase[1] (%)	Crop	Gizzard	Duodenum	Jejunum	Ileum	Ceca	Colon
0	0	5.08	3.33	6.09	5.93	6.73	5.85	6.63
	0.01	5.41	3.58	6.05	5.99	6.98	6.09	6.56
	0.1	5.08	3.51	6.07	6.01	6.87	5.75	6.52
30	0	5.56	3.33	6.06	5.91	7.05	5.99	6.99
	0.01	5.30	3.20	6.09	5.95	6.98	5.83	6.88
	0.1	5.18	3.48	6.18	5.96	7.08	5.77	6.69
60	0	5.29	3.54	6.05	5.99	7.08	6.02	6.92
	0.01	4.87	3.50	6.16	5.97	7.12	5.84	6.98
	0.1	5.23	3.26	6.19	6.01	7.26	6.04	7.17
SEM[2]		0.059	0.043	0.018	0.014	0.033	0.035	0.048
Main effects								
Hulless barley (%)								
0		5.19	3.47	6.07	5.98	6.86[b]	5.90	6.57[b]
30		5.35	3.34	6.11	5.94	7.03[a]	5.86	6.85[a]
60		5.13	3.43	6.13	5.99	7.15[a]	5.97	7.02[a]
BGase (%)								
0		5.31	3.40	6.07	5.94	6.95	5.95	6.85
0.01		5.19	3.43	6.10	5.97	7.02	5.92	6.80
0.1		5.16	3.42	6.15	5.99	7.07	5.85	6.79
ANOVA *P*-value								
Hulless barley		0.257	0.314	0.339	0.336	0.004	0.453	0.003
BGase		0.527	0.942	0.189	0.273	0.264	0.495	0.875
Hulless barley × BGase		0.114	0.057	0.487	0.838	0.367	0.070	0.361

[a,b]Means within a main effect or interaction not sharing a common superscript are significantly different ($P \leq 0.05$).
[1]BGase = β-glucanase.
[2]SEM = pooled SEM (n = 20 birds per treatment).

Table 8. Effects of hulless barley and β-glucanase on gastrointestinal tissue weights and lengths (proportional to body weight) of broiler chickens aged 28 d.

		Empty weight								Length						
HB[1] (%)	BGase (%)	Crop	Proven	Gizzard	Duo	Jejunum	Ileum	SI	Ceca	Colon	Duo	Jejunum	Ileum	SI	Ceca	Colon
0	0	0.28^{b}	0.40	1.14^{c,d}	0.75^{a,b}	1.40	0.91	3.05^{a,b}	0.35	0.13^{c,d}	1.67^{b}	4.09	3.89	9.95^{a,b}	1.58	0.38^{b}
	0.01	0.28^{b}	0.40	1.09^{c,d}	0.72^{a,b}	1.29	0.84	2.84^{a,b}	0.33	0.12^{d}	1.66^{b}	3.70	3.58	8.93^{c}	1.55	0.34^{b}
	0.1	0.30^{a,b}	0.37	1.06^{d}	0.74^{a,b}	1.37	0.91	3.01^{a,b}	0.37	0.13^{c,d}	1.69^{b}	3.92	3.79	9.39^{b,c}	1.59	0.38^{b}
30	0	0.31^{a,b}	0.38	1.24^{a,b,c}	0.73^{a,b}	1.28	0.93	2.94^{a,b}	0.36	0.14^{c,d}	1.74^{a,b}	4.07	3.98	9.78^{a,b,c}	1.66	0.41^{a,b}
	0.01	0.28^{b}	0.38	1.19^{b,c,d}	0.71^{b}	1.29	0.90	2.89^{a,b}	0.37	0.13^{c,d}	1.74^{a,b}	4.03	4.01	9.76^{a,b,c}	1.69	0.37^{b}
	0.1	0.28^{b}	0.38	1.24^{a,b,c}	0.73^{a,b}	1.25	0.85	2.83^{b}	0.39	0.14^{c,d}	1.81^{a,b}	3.93	3.96	9.70^{a,b,c}	1.69	0.40^{a,b}
60	0	0.34^{a}	0.38	1.19^{b,c,d}	0.72^{a,b}	1.37	1.00	3.03^{a,b}	0.36	0.17^{a,b}	1.72^{a,b}	4.21	4.18	10.25^{a,b}	1.67	0.41^{a,b}
	0.01	0.28^{b}	0.40	1.38^{a}	0.80^{a}	1.35	0.98	3.13^{a}	0.39	0.18^{a}	1.95^{a}	4.39	4.32	10.64^{a}	1.83	0.45^{a}
	0.1	0.29^{a,b}	0.38	1.32^{a,b}	0.73^{a,b}	1.30	0.91	2.94^{a,b}	0.37	0.15^{b,c}	1.75^{a,b}	4.01	4.11	10.07^{a,b}	1.69	0.39^{a,b}
SEM[2]		0.003	0.005	0.015	0.007	0.012	0.010	0.019	0.006	0.002	0.017	0.042	0.041	0.077	0.017	0.005
Main effects																
HB (%)																
0		0.29	0.39	1.10	0.74	1.35^{a}	0.89^{b}	2.97	0.35	0.13	1.67	3.90^{b}	3.76^{c}	9.42	1.57^{b}	0.37
30		0.29	0.38	1.22	0.72	1.28^{b}	0.89^{b}	2.89	0.37	0.14	1.76	4.01^{a,b}	3.98^{b}	9.75	1.68^{a}	0.39
60		0.31	0.39	1.30	0.75	1.34^{a,b}	0.97^{a}	3.03	0.37	0.16	1.81	4.21^{a}	4.21^{a}	10.32	1.73^{a}	0.42
BGase (%)																
0		0.31	0.39	1.19	0.73	1.35	0.95	3.01	0.36	0.15	1.71	4.13	4.02	9.99	1.64	0.40
0.01		0.29	0.39	1.22	0.74	1.31	0.91	2.95	0.37	0.14	1.78	4.04	3.97	9.78	1.69	0.39
0.1		0.29	0.38	1.20	0.73	1.31	0.89	2.93	0.38	0.14	1.75	3.95	3.95	9.72	1.66	0.39
ANOVA *P*-value																
HB		0.159	0.824	0.001	0.227	0.013	0.002	0.099	0.166	0.001	0.004	0.009	0.001	<0.001	0.005	0.003
BGase		0.005	0.565	0.523	0.857	0.217	0.069	0.236	0.400	0.117	0.238	0.212	0.774	0.262	0.491	0.629
HB × BGase		0.038	0.883	0.009	0.043	0.206	0.193	0.040	0.431	0.002	0.032	0.099	0.262	0.020	0.272	0.019

[a-d]Means within a main effect or interaction not sharing a common superscript are significantly different ($P \leq 0.05$).
[1]HB, hulless barley; Bgase, β-glucanase; Proven, proventriculus; Duo, duodenum; SI, small intestine.
[2]SEM = pooled standard error of mean (n = 20 birds per treatment).

Table 9. Effects of hulless barley and β-glucanase on the gastrointestinal content and organ weights as a percentage of the BW of broiler chickens aged 28 d.

		Content									Weight		
HB[1] (%)	BGase (%)	Crop	Proven	Gizzard	Duo	Jejunum	Ileum	SI	Ceca	Colon	Liver	Spleen	Pancreas
0	0	0.64	0.07	0.89^{b,c}	0.11	0.98	0.80	1.88	0.26	0.13	2.65	0.11	0.22
	0.01	0.52	0.09	0.65^{c}	0.10	0.89	0.80	1.78	0.27	0.12	2.51	0.11	0.21
	0.1	0.42	0.04	0.73^{b,c}	0.13	0.82	0.83	1.79	0.26	0.13	2.51	0.10	0.20
30	0	0.56	0.05	1.00^{a,b,c}	0.09	0.93	0.82	1.83	0.31	0.15	2.42	0.10	0.22
	0.01	0.37	0.05	1.14^{a,b}	0.07	0.79	0.80	1.65	0.32	0.13	2.56	0.11	0.24
	0.1	0.41	0.03	1.06^{a,b,c}	0.09	0.64	0.75	1.47	0.25	0.12	2.41	0.10	0.22
60	0	0.40	0.03	0.93^{b,c}	0.09	1.04	1.17	2.29	0.30	0.19	2.40	0.10	0.24
	0.01	0.71	0.05	1.46^{a}	0.06	0.89	1.06	2.01	0.26	0.19	2.57	0.10	0.26
	0.1	0.52	0.03	1.14^{a,b}	0.07	0.74	0.90	1.69	0.24	0.16	2.50	0.09	0.23
SEM[2]		0.054	0.005	0.037	0.004	0.020	0.023	0.038	0.010	0.005	0.020	0.002	0.003
Main effects													
HB (%)													
0		0.53	0.06	0.76	0.11^{a}	0.90^{a}	0.81^{b}	1.82^{a,b}	0.26	0.13^{b}	2.56	0.11	0.21^{c}
30		0.45	0.04	1.07	0.08^{b}	0.79^{b}	0.79^{b}	1.65^{b}	0.30	0.13^{b}	2.47	0.10	0.23^{b}
60		0.54	0.04	1.17	0.07^{b}	0.79^{b}	1.04^{a}	2.00^{a}	0.27	0.18^{a}	2.49	0.10	0.25^{a}
BGase (%)													
0		0.53	0.05	0.94	0.10	0.98^{a}	0.93	2.00^{a}	0.29	0.15	2.49	0.10	0.23^{a,b}
0.01		0.53	0.06	1.08	0.08	0.86^{b}	0.89	1.81^{a,b}	0.29	0.15	2.55	0.11	0.24^{a}
0.1		0.45	0.03	0.98	0.10	0.73^{c}	0.83	1.65^{b}	0.25	0.14	2.48	0.10	0.22^{b}
ANOVA *P*-value													
HB		0.564	0.140	<0.001	<0.001	0.019	<0.001	0.001	0.417	<0.001	0.129	0.340	<0.001
BGase		0.696	0.120	0.219	0.400	<0.001	0.118	0.001	0.177	0.392	0.254	0.460	0.001
HB × BGase		0.594	0.802	0.009	0.283	0.739	0.132	0.240	0.542	0.593	0.060	0.698	0.447

[a–c]Means within a main effect or interaction not sharing a common superscript are significantly different ($P \leq 0.05$).
[1]HB = hulless barley; BGase = β-glucanase; Proven = proventriculus; Duo = duodenum; SI = small intestine.
[2]SEM = pooled SEM (n = 20 birds per treatment).

main effects was significant. However, Mp and MW-10% was lower in the treatments with HB than wheat. The lower molecular weight parameters observed for HB are contradictory to the concept that HB contains high molecular weight β-glucan compared with wheat (Biliaderis and Izydorczyk, 2007). In contrast to the present study, previous research in our laboratory using similar diets has found that the molecular weight of ileal digesta soluble β-glucan was consistently higher in the birds fed HB than wheat (Karunaratne, 2020). Both previous and present studies used the same HB cultivar (CDC Fibar), and the dietary fiber content, including β-glucan, was approximately similar. However, the HB samples were grown in different years under different growing conditions that could have affected the structure and molecular weight of β-glucan in the grain (Tiwari and Cummins, 2009). The low molecular weight β-glucan of HB in the present study might be due to the activation of the endogenous BGase present in HB due to high-moisture weather conditions before harvest or during storage. It is well-established that water treatment improves the nutritive value of grains, including barley (Fry et al., 1958; Lepkovsky and Furuta, 1960), possibly by activating endogenous nonstarch polysaccharidases, which reduce the molecular weight of water-soluble nonstarch polysaccharides, including β-glucan. The endogenous enzyme activation in the grain is further supported by the low β-glucan molecular weight values of the ileal digesta in the present study compared with the molecular weight of barley grain (Wang et al., 2016) when the birds were fed HB-based diet, even without adding BGase. The lower β-glucan molecular values in the ileal digesta than in the diets were also noted for broiler chickens fed similar HB-based diets (without BGase) in our laboratory (Karunaratne, 2020).

Beta-glucanase decreased all three molecular weight parameters in the ileal digesta of chickens, supporting the conclusion that BGase depolymerizes high molecular weight β-glucan in the digestive tract of broiler chickens. The effect of BGase on MW-10% was significant at all three HB levels in the diet, and MW-10% is the most critical molecular weight criterion in the current research because it demonstrates the proportion of low molecular weight β-glucan, which might have the potential to increase carbohydrate fermentation. In addition, 0.1% BGase breaks down β-glucan into smaller molecules more than 0.01% BGase, according to Mp and Mw data, which confirms an enzyme dosage effect on the molecular weight reduction. The ileal viscosity was lower with the addition of BGase, which agrees with the β-glucan molecular weight distribution in the ileal digesta. Furthermore, the degree of BGase-associated average molecular weight reduction (indicated by the Mw) was higher in HB-based treatments than wheat-based diets, which was also observed in broiler chickens (coccidiosis-challenged) and laying hens given similar wheat and HB-based diets (Karunaratne, 2020). It might be

Table 10. Effects of hulless barley and β-glucanase on production performance of broiler chickens.

		BWG (g)					FI (g)					F:G				
HB[1] (%)	BGase (%)	d 0-7	d 7-14	d14-21	d21-28	d0-28	d0-7	d7-14	d14-21	d21-28	d0-28	d0-7	d7-14	d14-21	d21-28	d0-28
0	0	140^{a}	306^{a}	532^{ab}	704	1682^{a}	167	400	722^{a,b}	1032	2320^{a,b}	1.19^{b}	1.31^{c}	1.36	1.47	1.39
	0.01	143^{a}	318^{a}	542^{a}	720	1723^{a}	165	409	730^{a,b}	1039	2342^{a,b}	1.15^{b}	1.29^{c}	1.35	1.45	1.36
	0.1	135^{a,b}	311^{a}	525^{a,b}	705	1676^{a}	157	399	704^{a,b}	1010	2254^{b}	1.16^{b}	1.28^{c}	1.34	1.43	1.35
30	0	138^{a}	297^{a,b}	521^{a,b}	685	1645^{a,b}	162	393	713^{a,b}	1007	2256^{a,b}	1.17^{b}	1.31^{c}	1.38	1.48	1.40
	0.01	140^{a}	314^{a}	537^{a}	690	1680^{a}	165	419	733^{a}	1024	2325^{a,b}	1.18^{b}	1.34^{b,c}	1.37	1.49	1.39
	0.1	135^{a,b}	309^{a}	525^{a,b}	691	1659^{a,b}	158	403	715^{a,b}	1004	2280^{a,b}	1.17^{b}	1.31^{c}	1.36	1.46	1.38
60	0	143^{a}	303^{a,b}	507^{a,b,c}	698	1649^{a,b}	167	421	729^{a,b}	1055	2371^{a}	1.17^{b}	1.39^{a,b}	1.44	1.53	1.45
	0.01	127^{b}	280^{b}	473^{c}	655	1535^{c}	160	404	687^{b}	1004	2254^{b}	1.26^{a}	1.45^{a}	1.45	1.53	1.47
	0.1	126^{b}	296^{a,b}	498^{b,c}	656	1575^{b,c}	157	403	705^{a,b}	1004	2284^{a,b}	1.26^{a}	1.35^{b,c}	1.42	1.54	1.43
SEM[2]		1.03	2.20	3.58	4.57	9.22	0.93	2.46	3.72	5.27	2.46	0.01	0.01	0.01	0.01	0.01
Main effects																
HB (%)																
0		140	312	533	710^{a}	1694	163	403	719	1027	2311	1.17	1.29	1.35^{b}	1.45^{b}	1.37^{c}
30		138	307	528	688^{a,b}	1662	162	405	720	1012	2297	1.17	1.32	1.37^{b}	1.47^{b}	1.39^{b}
60		132	293	493	670^{b}	1586	161	408	707	1021	2292	1.23	1.40	1.44^{a}	1.53^{a}	1.45^{a}
BGase (%)																
0		140	305	520	696	1659	165^{a}	405	721	1031	2316	1.18	1.34	1.39	1.49	1.42^{a}
0.01		137	304	517	688	1646	163^{a}	411	717	1022	2312	1.20	1.36	1.39	1.49	1.41^{a}
0.1		132	302	516	684	1637	157^{b}	400	708	1006	2272	1.19	1.31	1.37	1.48	1.39^{b}
ANOVA *P*-value																
HB		0.001	0.003	0.001	0.001	0.001	0.705	0.621	0.216	0.463	0.693	0.001	0.001	0.001	0.001	0.001
BGase		0.004	0.749	0.852	0.513	0.478	0.002	0.233	0.279	0.122	0.094	0.025	0.006	0.214	0.401	0.002
HB × BGase		0.001	0.006	0.024	0.132	0.004	0.349	0.054	0.027	0.187	0.008	0.001	0.044	0.817	0.766	0.150

[a-c]Means within a main effect or interaction not sharing a common superscript are significantly different ($P \leq 0.05$).
[1]HB, hulless barley; Bgase, β-glucanase; BWG, body weight gain; FI, feed intake; F:G, feed to gain ratio.
[2]SEM = pooled standard error of mean (n = 10 cages per treatment).

associated with the differences in the β-glucan structure between these grains; specifically, the ratio of cellotriosyl-to-cellotetraosyl units (DP3-to-DP4 ratio) in wheat and barley β-glucan, which were 3.0–4.5 and 2.3–3.4, respectively. Higher and lower DP3-to-DP4 ratios of β-glucan result in a higher predominant molar proportion. Wheat has a higher predominant β-glucan molar proportion than barley (DP3; 67–72%, DP4; 21–24% in wheat and DP3; 52–69, DP4; 25–33% in barley) that results in a more regular and uniform β-glucan structure (Biliaderis and Izydorczyk, 2007). Increased aggregation and lower solubility of β-glucan have been reported in β-glucan containing a higher predominant molar proportion (Burton and Fincher, 2014), and this might be the reason for β-glucan showing a lower susceptibility to exogenous BGase in wheat than barley. The effect of 0.1% BGase on AME_n is not different when comparing wheat- and barley-fed chickens because a smaller degree of β-glucan molecular weight reduction might be adequate to reduce the digesta viscosity and increase energy utilization, despite the lower BGase susceptibility in wheat β-glucan.

Nitrogen-corrected AME values for both wheat- and HB-based diets were lower than the calculated value shown in Table 1. Several factors might be responsible for the lower energy, including different growing conditions that affect the grain nutrient content (Bedford et al., 1998; Scott et al., 1998; Ball et al., 2013). However, the decrease in AME_n with increasing levels of HB is likely due to the characteristics of the grains. The HB sample used in the current research contained more fiber and less starch than the wheat sample (HB: TDF 29.0%; starch; 49.7%, wheat: TDF; 15.2%, starch; 64.1%); this finding is similar to previous research (Biliaderis and Izydorczyk, 2007; Dhingra et al., 2012). These chemical analyses indicate the approximately similar nutrient composition of HB and wheat, which was an assumption regarding the diet formulation, was not accurate in the present study. Overall, BGase increased AME_n of broiler chickens fed an HB-based diet, and it is significant at the 60% HB level. The increased AME_n can be attributed to the increased energy derived from increased digestibility of nutrients as a result of low digesta viscosity, which resulted from the depolymerization of high molecular weight β-glucan (Classen et al., 1985). However, the ileal viscosity in the present study was not higher in birds fed HB- than wheat-based diets, although HB contained a very high percentage of TDF (29.0%) and total β-glucan (8.7%). Furthermore, SDF was also high in HB compared with wheat (9.4 vs. 1.6%). Also contrary to the lower digesta viscosity for HB was the higher in vitro viscosity of HB (49.3 cP) than that of wheat (1.7 cP). Previous studies, however, have shown that in vitro viscosity of ingredients does not always reflect viscosity in the digestive tract (Dikeman et al., 2006). The smaller difference of the ileal viscosity between broilers fed wheat- and HB-based diets might be due to the endogenous BGase activation of HB in the digestive tract of chickens (Ribeiro et al., 2011), which results in low molecular weight β-glucan that attributed to a low ileal viscosity in the broiler chickens. A high level of arabinoxylan in wheat might also increase the ileal viscosity in wheat-fed broilers (Choct and Annison, 1992; Kiarie et al., 2014). The soluble, viscous β-glucan is more likely to be digested by small intestinal microbes than soluble, viscous arabinoxylan (Karppinen et al., 2000). Therefore, the minimal amount of viscous arabinoxylan in wheat might have been persisted and created a higher digesta viscosity than the viscous and plentiful but highly labile β-glucan in HB. The soluble β-glucan content, which is the main component that affects viscosity, was not analyzed in the present study, although it is generally higher in barley than wheat (Henry, 1985). Beta-glucanase reduced the ileal viscosity in the broilers fed wheat- and barley-based diets, which corresponds with the β-glucan molecular weight reduction in the ileal digesta. In addition to the decline of digesta viscosity, nutrient digestibility increases with the elimination of nutrient encapsulation by the cell wall (Hesselman and Åman, 1986) because of the activity of nonstarch polysaccharidases in the diets and thereby increases the AME. Beta-glucanase also modifies the microbial profile in the gut and may affect nutrient digestibility that results in a higher AME_n in the birds given barley-based diets (Mathlouthi et al., 2002; Józefiak et al., 2010).

Short-chain fatty acid levels and gastrointestinal pH were assessed to investigate treatment effects on carbohydrate fermentation in broiler chickens because the present study hypothesized carbohydrate fermentation would increase because of higher levels of low molecular weight β-glucan in ileal digesta. Both SCFA and pH data fail to support this hypothesis. Short-chain fatty acid and pH levels were unaffected by dietary treatments shown to produce low molecular weight β-glucan. The only significant pH effects were higher values for ileum and colon pH when HB was fed rather than wheat. The fewer treatment effects on SCFA levels and pH were unexpected because higher HB content in diets increased substrate (SDF) for fermentation in the lower digestive tract, potentially lowering intestinal pH by increased SCFA production. Furthermore, there was no treatment effect on the cecal pH. Intestinal pH is not only an indication of SCFA concentration but is also influenced by diet composition such as minerals and proteins, and endogenous secretions in the digestive tract. Protein fermentation in the lower GIT results in the production of ammonia, biogenic amines, indoles, and phenols as well as SCFA, and these fermentation products may increase digesta pH (Apajalahti, 2005). Minerals in feed ingredients buffer hydrogen ions resulting from SCFA and thereby increase the pH in the digesta of chickens (Heller and Penquite, 1936; Shafey et al., 1991; Pang and Applegate, 2007).

As noted above, there were no treatment effects on broiler ileal and cecal SCFA concentrations in the current research, which agrees with previous research based on barley feeding in broilers that observed small and inconsistent dietary BGase effects (Józefiak et al., 2005, 2006). The cecal propionic acid and total SCFA concentrations were increased with BGase in one study

(Józefiak et al., 2005), whereas another study found no BGase effect on the concentrations of SCFA in chickens (Józefiak et al., 2006). Like the present study, BGase affected ileal and cecal SCFA with no clear trends in coccidiosis-challenged broilers given similar HB-based diets; an exception was 0.1% BGase, which increased all SCFA compared with 0.01% BGase at day 11 (Karunaratne, 2020). The ileal and cecal concentrations of SCFA depend on many factors, including the availability of fermentable substrates, SCFA production, and the absorption that depends on the mechanism of SCFA transport and the expression of transporters involved with the mechanisms (Tan et al., 2014). Therefore, SCFA levels may not be an accurate indicator of carbohydrate fermentation in the present study, considering the aforementioned factors and the time of sample collection affecting the variability of ileal and cecal evacuation.

Short-chain fatty acids have been shown to increase peptide tyrosine-tyrosine (**PYY**), glucagon-like peptide-1 (**GLP-1**), and glucagon-like peptide-2 (**GLP-2**) production from L-cells in the mammalian small intestine (Keenan et al., 2006; Brooks et al., 2017). In turn, PYY and GLP-1 molecules activate the ileal brake, which decreases gastric emptying and gastrointestinal motility (Meyer et al., 1998; Maljaars et al., 2008), whereas GLP-2 is involved in mucosal hyperplasia of the intestines (Tsai et al., 1997; Hu et al., 2010). The importance of digestive tract microbiota and potentially fermentation products in ileal brake activation is shown by the increased secretion of PYY and GLP-1 in conventional compared with germ-free mice fed a high β-glucan diet (Miyamoto et al., 2018). In chickens, these gut hormones and the L-cell activators appear to decrease the FI (Furuse et al., 1997; Aoki et al., 2017; Herwig et al., 2019) and increase gut development (Herwig et al., 2020), which is an indication of reduced gut motility and emptying. The effect of HB on increasing the relative empty weights, lengths, and content weights of the digestive tract sections in the present study could be attributed to the PYY-induced and GLP-1–induced reduction of gut motility and gastric emptying that leads to lower nutrient digestibility. The growth of the intestine is associated with GLP-2 secretion and might contribute to the increased intestinal empty weight and length, and consequently increase the content it holds.

Feeding HB decreases nutrient digestibility via a number of mechanisms including increased digesta viscosity (Pettersson and Åman, 1989; Bedford et al., 1991), nutrient encapsulation (Kocher et al., 2003; Khadem et al., 2016), and an altered digestive tract microbiota (Choct and Annison, 1992). To compensate for the reduced digestibility, the digestive tract size and pancreas weight increase as observed in the current and previous research (Brenes et al., 1993; Karunaratne, 2020). The addition of BGase improves nutrient digestibility and decreases the need for compensation by the digestive tract. In the current work, pancreas weights decreased with enzyme addition because the requirement of digestive enzymes was lower because of an improvement in diet digestibility.

The gizzard content increased with the addition of HB to the diets, and it demonstrates the necessity of increased gizzard retention time of digesta to complete the grinding of the high fiber content in HB. The fiber composition of this grain confounds the effect of HB on the gizzard content. Activation of the ileal brake could reduce gastric emptying, which is related to the soluble fraction of β-glucan. The empty gizzard weight was also higher in the birds fed HB- than wheat-based diets, and therefore, the larger gizzard might hold more content and increased the content weight. The content weights of the duodenum and jejunum decreased with HB, and it might be associated with high insoluble fiber in HB, which increase the digesta passage rate (Hetland and Svihus, 2001). However, HB increased the ileum and ceca content weights, and it is probably due to the solubilization of the insoluble fiber, which increases the feed retention time in the lower digestive tract of broiler chickens (Salih et al., 1991; Almirall and Esteve-Garcia, 1994). Beta-glucanase effect was significant on the jejunum and small intestine content, and the reduction of the content is associated with the depolymerization of β-glucan that reduces digesta viscosity and thereby increases the feed passage rate (Almirall and Esteve-Garcia, 1994).

The production parameters were within the normal range of Ross 308 Broiler Performance Objectives when considering the day 0–28 production cycle (Aviagen, 2014). The interactions between the main effects were significant on the BWG and FI, but the differences were mostly minor. Overall, HB reduced the growth performance, and it is associated with the high fiber and lower nutrient content, including starch in HB, compared with wheat in the present study (HB: TDF 29.0%; starch; 49.7%, wheat: TDF; 15.2%, starch; 64.1%), which affects nutrient digestibility, including AME_n. The negative effects associated with the fiber, including β-glucan in HB, eventually affect production performance in broilers (Mathlouthi et al., 2002; Rodríguez et al., 2012; Jacob and Pescatore, 2014). The difference in AME_n between wheat and HB might have also affected bird growth performance. The BGase effect on the growth performance of broiler chickens was age-dependent in the present study. The BWG and feed efficiency decreased with the addition of BGase to 60% HB-based diets from day 0 to 7, and it might be associated with the less mature gut microbiota in the broilers at a young age, which cannot utilize the high amount of low molecular weight carbohydrates released by the activity of dietary BGase (Bautil et al., 2019). However, BGase did not affect the BWG and F:G after day 7 but improved the growth performance when considering the entire period, which can be explained by the increasing ability of intestinal microbes to utilize non-starch polysaccharides as they adapt to a high fiber containing diet and increased production of fiber-degrading enzymes, including BGase (Lee et al., 2017; Bautil et al., 2019). This age-dependent effect of

BGase on the performance was also observed in the coccidiosis-challenged broiler chickens given similar HB-based diets in a previous study (Karunaratne, 2020) with more significant effects, which is possibly due to the disease challenge associated modification of gut microbiota.

In conclusion, exogenous BGase depolymerized high molecular weight β-glucan in the digestive tract of broiler chickens. The BGase dosage effect was also significant for digesta soluble β-glucan depolymerization. The resulting low molecular weight β-glucan was not able to increase the carbohydrate fermentation in the ileum and ceca, as estimated by digesta SCFA levels and pH. Hull-less barley increased the digestive tract size and content, whereas BGase decreases these parameters for the most part. Overall, HB reduced broiler performance. Beta-glucanase decreased the performance of young birds fed higher levels of HB, whereas having no effect or improving feed efficiency at 0.1% level in older birds or for the entire length of the experiment, respectively.

ACKNOWLEDGMENTS

The authors would like to acknowledge the National Science and Engineering Research Council (NSERC) Industrial Research Chair Program for financial support for this project. Funding for this program was derived from Aviagen, Canadian Poultry Research Council, Chicken Farmers of Saskatchewan, NSERC, Ontario Poultry Industry Council, Prairie Pride Natural Foods Ltd., Saskatchewan Egg Producers, Saskatchewan Hatching Egg Producers, Saskatchewan Turkey Producers, Sofina Foods Inc., and the University of Saskatchewan. The technical support given by Dawn Abbott, Tracy Exley, and the Poultry Centre staff at the University of Saskatchewan is also acknowledged.

DISCLOSURES

We declare that we have no financial and personal relationships with other people or organizations that can inappropriately influence our work and there is no professional or other personal interest of any nature or kind in any product, service, and/or company that could be construed as influencing the content of this article. Please note one author, M.R.B., works for the company that manufactures an enzyme used in this study, but he did not inappropriately influence this work.

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