Hulless barley and beta-glucanase levels in the diet affect the performance of coccidiosis-challenged broiler chickens in an age-dependent manner

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ABSTRACT Diet β-glucanase (**BGase**) depolymerizes viscous β-glucan into lower molecular weight carbohydrates, which might act as a prebiotic in chickens exposed to enteric disease. Coccidiosis-challenged broiler chickens were fed graded levels of hulless barley (**HB**) and BGase to determine their effects on growth performance. Broilers were fed high β-glucan HB (CDC Fibar; 0, 30, and 60% replacing wheat) and BGase (Econase GT 200P; 0, 0.01, and 0.1%) in a 3 × 3 factorial arrangement. A total of 5,346 broilers were raised in litter floor pens and vaccinated for coccidiosis in feed and water on day 5. Each treatment was assigned to 1 pen (66 birds) in each of 9 rooms. Statistical significance was set at $P \leq 0.05$. Overall, HB decreased body weight gain (**BWG**) and increased feed: gain ratio (**F:G**) of broilers. From day 0 to 11, BGase did not affect BWG and F:G, at the 0 and 30% HB. However, at 60% HB, the 0.01% BGase improved them, and the 0.1% BGase had no effect on BWG and increased F:G. For the day 22 to 32 and 0 to 32 periods, BGase did not affect BWG for 0 and 30% HB levels, but for the 60% HB, both BGase levels increased gain. The 0.1% level of BGase resulted in the lowest F:G for all HB levels, with the degree of response increasing with HB. No interaction was found for ileal digesta viscosity at day 11; the level of HB did not affect viscosity, but both levels of BGase decreased viscosity. At day 33, BGase did not affect viscosity at 0 and 30% HB levels, but viscosity was lowered for the 0.1% BGase treatment at the 60% HB level. In conclusion, HB reduced broiler performance, and BGase alleviated most but not all the effects. In young birds fed 60% HB, 0.1% BGase did not impact BWG and increased F:G.

Key words: beta-glucan, prebiotic, non-starch polysaccharide, feed enzyme, viscosity

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INTRODUCTION

The reduction in the use of in-feed antibiotics has made an investigation of alternatives to antibiotics a major research priority. Probiotics, prebiotics, essential oils, volatile fatty acids (e.g., butyric acid), and feed enzymes (e.g., nonstarch polysaccharidases) are some of the alternatives to antibiotics that are being used or studied in poultry production (Ducatelle et al., 2015; Gadde et al., 2017). For a complete understanding of the efficacy of alternatives, testing in chickens undergoing a disease challenge is essential so that their ability to alleviate adverse infection effects can be more clearly delineated.

The use of exogenous enzymes, especially nonstarch polysaccharidases, in poultry feed has been suggested as an alternative to antibiotics because many previous studies demonstrated beneficial effects of these enzymes on performance and gut health parameters in chickens (Bedford, 2019). Xylanase supplementation in poultry feed has received the most attention because arabinoxylans are commonly found in the cell walls of predominant cereal grains (wheat and maize) used in the poultry industry. Xylanase increases the growth performance of broiler chickens through reducing digesta viscosity induced by soluble arabinoxylan (Choct et al., 2004), and by cell wall hydrolysis that reduces nutrient encapsulation (Bedford and Autio, 1996; Ravn et al., 2018). Furthermore, exogenous xylanase increases the concentration of digesta arabinoxylo-oligosaccharides in the broilers fed wheat-based diets (Morgan et al., 2017).

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The resulting arabinoxylo-oligosaccharides beneficially modulate digestive tract microbiota and the epithelial integrity through increasing microbial fermentation of these low molecular weight (**MW**) carbohydrates (De Maesschalck et al., 2015; Lee et al., 2017). The overall positive effects of exogenous xylanase on performance and gut health in chickens have led to the investigation of other nonstarch polysaccharide degrading enzymes, including β-glucanase (**BGase**) in poultry research.

It is a common practice to use exogenous BGase in barley-based poultry feed to reduce β-glucan induced digesta viscosity (Salih et al., 1991; Fuente et al., 1995) and mitigate the negative effects associated with viscosity. It is supported by the positive effect of BGase on nutrient digestibility (Hesselman and Åman, 1986; Edney et al., 1989), apparent metabolizable energy (Potter et al., 1965; Perttilä et al., 2001) and bird performance (Classen et al., 1988; Campbell et al., 1989). However, little research investigated the effect of BGase on growth performance in different age categories of broiler chickens fed barley-based diets (Salih et al., 1991; Józefiak et al., 2005, 2006). Moreover, most of the studies on enzyme use in barley diets have used mixed enzyme sources (at least BGase and xylanase activities), and there is minimal research using purified feed BGase to study the performance of broiler chickens (Dos Santos et al., 2013). The use of a purified BGase in the present study contributes to understanding the single effect of β-glucan rather than a combination of nonstarch polysaccharides on bird performance, probably acting as fermentable substrates for digestive tract microbiota. Although the effect of BGase on reducing digesta viscosity is well-established, the evaluation of BGase dose on broiler performance is essential to determine if increasing levels of BGase can achieve a higher growth performance, possibly through increasing microbial fermentation in the digestive tract of broilers fed barley-based diets.

The objective of the study was to evaluate the effects of hulless barley (**HB**) and BGase levels on the growth performance of broiler chickens under a coccidiosis challenge at different age groups. It was hypothesized HB would decrease broiler performance, whereas exogenous BGase will increase the performance in a dose-dependent manner in broilers of all age groups fed HB-based diets.

MATERIALS AND METHODS

The experimental procedure was approved by the Animal Research Ethics Board of the University of Saskatchewan and adhered 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 5,346 1-day-old male and female (Ross × Ross 308) broiler chickens were obtained from a commercial hatchery and randomly placed (33 males and 33 females per pen) in 81 floor pens (2.3 m length and 2 m width) in 9 environmentally controlled rooms with an estimated trial and density of 31 kg/m^2. There were 9 floor pens in each room. Each of the 9 dietary treatments was randomly assigned to 1 pen per room, providing 9 replications per treatment. An equal amount of straw was placed in each room with a 7.5 to 10 cm thickness. Room temperature was 33°C on day 0, and then gradually decreased until it was 21°C by day 25. Day length was 23 h at the beginning of the trial, and it was gradually reduced to 17 h by day 12. Light intensity was 20 lux at the start of the trial and gradually reduced to 10 lux by day 10. Each pen was equipped with a tube feeder having a pan diameter of 36 (0–25 d) or 43 cm (>25 d) to provide ad libitum feed. Each pen was provided with a height-adjustable nipple drinker, each having 6 Lubing nipples. Supplementary feed and water were provided to each pen during the first week using a cardboard egg tray and an ice cube tray (16 cells).

Experimental Diets

Treatments were arranged in a 3 × 3 factorial design based on diet HB (CDC Fibar; 0, 30, and 60%) and BGase (Econase GT 200 P from ABVista, Wiltshire, UK; 0, 0.01, and 0.1%) levels. The BGase activities in diets were calculated to be 0, 20,000, and 200,000 BU/kg for the 0, 0.01, and 0.1% levels, respectively. Hulless barley (CDC Fibar; β-glucan content–8.7%) replaced wheat in each experimental diet; HB and wheat were assumed to have approximately the same nutrient composition. Starter diets were fed from day 0 to 11 and grower diets after that. The ingredients and calculated nutrient levels are presented in Table 1. Diets were formulated in accordance with Ross 308 broiler nutrition specifications (Aviagen, 2014). The starter diets were made in crumble form, and the grower diets were initially given in a crumble form, and then switched to a pellet form. The pelleting temperature was maintained between 70°C and 75°C for all diets to prevent BGase inactivation. Beta-glucanase (EC 3.2.1.6) and xylanase activities (EC 3.2.1.8) of the diets were analyzed in accordance with the AB Vista methods of ESC Standard Analytical Method SAM042-01 and SAM038, respectively. Xylanase activity was nondetectable in the diets, and BGase activity approximated the expected enzyme activity values.

Coccidiosis Challenge

All the birds were challenged with Coccivac B-52 live vaccine (Merck Animal Health, Madison, NJ), which contains *Eimeria acervulina, Eimeria mivati, Eimeria maxima* (2 strains), and *Eimeria tenella* oocysts. Vaccination (1.3 × recommended dose) was completed at 5 d of age to facilitate uniform oocyst intake by spraying diluted vaccine (1,000 doses in 500 mL distilled water) onto 1 egg tray containing feed and 1 ice cube tray containing water in each pen. Feeders and drinkers were raised in each pen (to prevent bird access) before starting vaccination and kept up until the vaccine containing

Table 1. Ingredients and calculated nutrient levels of starter and grower diets (%, as-is basis).

Item	Starter (Day 0–11)	Grower (Day 11–33)
Ingredient		
Cereal grain (wheat and hulless barley)[1]	59.09	64.80
Soybean meal	32.97	26.93
Canola oil	3.29	4.03
Monodicalcium phosphate	1.40	1.20
Limestone	1.64	1.52
Sodium chloride	0.43	0.38
Vitamin-mineral broiler premix[2]	0.50	0.50
Choline chloride	0.10	0.10
DL-Methionine	0.30	0.27
L-Threonine	0.07	0.05
L-Lysine HCl	0.21	0.22
Nutrient, calculated		
AME (kcal/kg)	3,000	3,100
Crude protein	23.46	21.24
Crude fat	4.74	5.57
Calcium	0.96	0.87
Chloride	0.38	0.36
Nonphytate phosphorous	0.48	0.44
Potassium	0.92	0.83
Sodium	0.20	0.18
Digestible arginine	1.50	1.35
Digestible isoleucine	0.90	0.81
Digestible leucine	1.61	1.47
Digestible lysine	1.28	1.15
Digestible methionine	0.60	0.54
Digestible methionine and cysteine	0.95	0.87
Digestible threonine	0.86	0.77
Digestible tryptophan	0.27	0.24
Digestible valine	0.96	0.87

[1]Wheat: total dietary fiber (TDF), 14.4; insoluble dietary fiber (IDF), 12.4; soluble dietary fiber (SDF), 2.0; β-glucan, 0.64/hulless barley: TDF, 26.7; IDF, 18.9; SDF, 7.8; β-glucan, 8.70 (% DM basis).

[2]Vitamin-mineral premix provided the following per kilogram of 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.

supplementary feed and water were consumed. Therefore, birds only had access to vaccinated feed during that time. In addition, a Kraft brown paper strip (Model S-8511S; ULINE Canada, Milton, Ontario, Canada) of 30 cm width was placed under the full length of the nipple drinker line in each pen before the coccidiosis challenge to facilitate coprophagy and coccidian oocyst cycling. Humidity was kept high (60%) in the rooms via humidifiers and water spray application to litter to facilitate oocyst cycling.

Performance Data Collection

Performance parameters including feed intake (**FI**) and body weight were taken on a pen basis at day 11, 22, and 32. Body weight gain (**BWG**) and mortality corrected feed: gain ratio (**F:G**) were calculated. Mortality was recorded daily, and dead birds were sent to Prairie Diagnostic Services (University of Saskatchewan) for necropsy.

Sample Collection

At each collection day (day 11 and 33), 2 birds were selected from each pen and euthanized by injection of T-61 containing embutramide, mebezonium iodide, and tetracaine hydrochloride (Merck Animal Health, Kirkland, Quebec, Canada) into the brachial vein. Tissues were removed from the bird carcass, separated into different gastrointestinal tract (**GIT**) sections (crop, proventriculus, gizzard, duodenum, jejunum, ileum, ceca, and colon), and then full and empty weights and lengths (when appropriate) were recorded. Content weight was obtained by subtracting empty from the full weight. The liver, spleen, and pancreas were removed and weighed. Empty weights, lengths, content, and organ weights were divided by individual body weights to obtain relative weights of each parameter. The ileal contents were collected into plastic snap-cap vials and centrifuged at 17,013 × g at 40°C for 5 min using a Beckman microfuge (Model E348720; Beckmann instruments, INC, Palo Alto, CA). A Brookfield cone-plate viscometer (Model LVDV-III; Brookfield Engineering Labs, INC, Stoughton, MA) maintained at 40°C (40 rpm; shear rate 300 s^{-1}) was used to measure ileal supernatant viscosity.

Dietary Analysis

Experimental diets and ingredients (wheat and HB) were ground using a Retsch laboratory mill (Retsch ZM 200; Germany) to 1 mm (for the analysis of insoluble

Table 2. Analyzed chemical composition of the experiment diets (%, DM basis).

	0% hulless barley			30% hulless barley			60% hulless barley		
Item	0% BGase	0.01% BGase	0.1% BGase	0% BGase	0.01% BGase	0.1% BGase	0% BGase	0.01% BGase	0.1% BGase
Starter diets									
Total starch	38.5	36.3	35.1	35.2	35.2	35.1	34.2	31.9	32.5
Crude protein	25.5	25.5	26.7	26.1	26.4	27.1	26.3	26.9	25.3
Ether extract	3.7	3.4	4.0	3.6	4.1	4.1	4.2	3.8	4.5
Ash	5.6	5.7	6.1	6.2	5.6	6.2	6.1	6.3	6.7
Total dietary fiber	23.4	24.1	24.8	25.5	25.2	27.0	28.2	28.1	27.0
Insoluble dietary fiber	19.3	19.2	19.4	19.8	19.6	20.1	20.3	19.5	17.6
Soluble dietary fiber	4.1	4.9	5.3	5.7	5.7	6.9	7.9	8.6	9.3
Total β-glucan	1.0	0.9	0.8	2.9	2.9	2.5	5.0	4.6	4.9
Grower diets									
Total starch	40.1	36.8	38.7	35.9	36.1	36.9	33.9	33.7	33.2
Crude protein	23.1	24.1	22.9	25.2	24.3	24.6	25.8	24.7	25.2
Ether extract	4.9	4.6	4.6	4.7	4.4	5.0	4.5	5.1	5.1
Ash	5.6	5.7	5.6	6.1	5.6	6.1	6.0	6.5	6.0
Total dietary fiber	23.3	25.2	26.8	27.4	25.7	27.1	28.5	30.0	25.5
Insoluble dietary fiber	20.3	21.6	22.4	22.7	20.7	21.9	22.0	23.0	19.4
Soluble dietary fiber	3.0	3.4	4.5	4.8	4.9	5.2	6.5	6.9	6.2
Total β-glucan	0.6	1.0	0.9	2.3	2.5	2.6	4.4	4.4	4.3

and soluble dietary fiber, N, fat, and ash) and 0.5 mm (for the analysis of β-glucan and total starch) screen-hole sizes. Insoluble dietary fiber (**IDF**) and soluble dietary fiber (**SDF**) were analyzed using a Megazyme kit (total dietary fiber 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, 2010). The addition of IDF and SDF obtained total dietary fiber (TDF). Beta-glucan was analyzed (AOAC method 995.16 [AOAC, 2006], AACC method 32-23 [AACC, 2010], and ICC method 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). The total starch analysis was completed based on AOAC method 996.11 (AOAC, 2006) and AACC method 76-13.01 (AACC, 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 protein analyzer (Model Leco-FP-528L; Leco Corporation, St. Joseph, MA), and 6.25 was used as the N to CP conversion factor. Fat content was determined by ethyl ether extraction using Goldfish Extraction Apparatus (Labconco model 35001; Labconco, Kansas, MO) following the AOAC method 920.39 (AOAC, 2006). Ash content was analyzed according to AOAC method (AOAC, 2006) 942.05 using a muffle oven (Model Lindberg/Blue BF51842C, Asheville, NC). Moisture was analyzed using the AOAC method 930.15 (AOAC, 2006).

Statistical Analysis

The experiment was a randomized complete block design with a room used as a block to account for potential environmental differences between rooms. Data were analyzed using a 2-way analysis of variance of SAS 9.4 Proc mixed model to determine the main effects of, and interaction between, HB and BGase (SAS 9.4, Cary, NC, 2008; SAS Institute, 2008). The significance level was $P \leq 0.05$, and the trends were considered when $0.10 \leq P > 0.05$. Mean separation was completed using the Tukey-Kramer test. Data were tested for normality using the Shapiro-Wilk test and the percentage data were log-transformed when they were not normally distributed.

RESULTS

Ingredient Nutrient Content

The TDF, IDF, and SDF in HB were 26.7, 18.9, and 7.8%, respectively. In wheat, 14.4% TDF, 12.4% IDF, and 2.0% SDF were determined. Total β-glucan was analyzed as 8.70 and 0.64% in HB and wheat, respectively. Total starch, CP, fat, and ash were analyzed as 53.7, 16.2, 2.8, and 2.4%, respectively, in HB, whereas the corresponding values for wheat were 62.8, 14.9, 1.2, and 1.7%. The nutrient composition of the experimental diets was included in Table 2.

Performance Parameters

Dietary HB and BGase affected broiler performance in an age-dependent manner (Table 3). Production data were influenced by interactions between HB and BGase, excepting BWG from 11 to 22 d and FI from 22 to 32 d. Overall, BWG, FI, and F:G were poorer as the level of HB increased in diets. From 0 to 11 d, BWG, FI, and F:G were not affected by BGase level for the birds fed 0 and 30% HB. For the birds fed diets containing 60% HB, 0.01% BGase resulted in faster growth than broilers from either 0 or 0.1% BGase treatments. Similarly, F:G was lower for birds fed diets containing 0.01% BGase than the other 2 enzyme levels, but for this parameter, values for 0.1% BGase were higher (poorer) than the 0% BGase treatment. Feed intake from 0 to 11 d was higher for 0.01% BGase than for the other 2 enzyme levels

Table 3. Effects of hulless barley and β-glucanase on production performance of broiler chickens.

	Body weight gain (kg)				Feed intake (kg)				Feed to gain ratio			
Item	Day 0–11	Day 11–22	Day 22–32	Day 0–32	Day 0–11	Day 11–22	Day 22–32	Day 0–32	Day 0–11	Day 11–22	Day 22–32	Day 0–32
HB (%) × BGase (%)												
0 × 0	0.278^{a,b}	0.641	0.960^{a,b}	1.879^{a,b,c}	0.338^{a,b}	1.035^{a}	1.626	3.00^{a}	1.20^{e,f}	1.49^{b,c}	1.66^{c,d}	1.53^{c,d,e}
0 × 0.01	0.275^{a,b,c}	0.672	0.952^{a,b}	1.899^{a,b}	0.337^{a,b}	1.037^{a}	1.633	3.006^{a}	1.20^{e,f}	1.42^{c}	1.69^{c}	1.52^{d,e,f}
0 × 0.1	0.286^{a}	0.668	0.977^{a}	1.931^{a}	0.343^{a}	1.037^{a}	1.581	2.962^{a,b}	1.18^{f}	1.43^{c}	1.61^{d}	1.48^{f}
30 × 0	0.266^{b,c}	0.641	0.919^{b,c}	1.826^{c}	0.336^{a,b}	1.038^{a}	1.591	2.965^{a,b}	1.25^{c,d}	1.50^{b,c}	1.71^{c}	1.57^{c}
30 × 0.01	0.275^{a,b,c}	0.646	0.924^{b,c}	1.846^{b,c}	0.346^{a}	1.029^{a}	1.576	2.950^{a,b}	1.22^{c,d,e}	1.48^{c}	1.67^{c,d}	1.53^{c,d,e}
30 × 0.1	0.277^{a,b}	0.649	0.939^{a,b}	1.865^{b,c}	0.344^{a}	1.034^{a}	1.525	2.903^{b,c}	1.21^{d,e,f}	1.48^{c}	1.60^{d}	1.49^{e,f}
60 × 0	0.243^{d}	0.562	0.788^{e}	1.594^{e}	0.328^{b}	0.979^{b}	1.540	2.846^{c,d}	1.32^{b}	1.62^{a}	1.94^{a}	1.72^{a}
60 × 0.01	0.264^{c}	0.603	0.859^{d}	1.726^{d}	0.339^{a,b}	1.033^{a}	1.569	2.941^{a,b}	1.26^{c}	1.58^{a,b}	1.80^{b}	1.63^{b}
60 × 0.1	0.237^{d}	0.622	0.881^{c,d}	1.740^{d}	0.331^{b}	0.982^{b}	1.499	2.813^{d}	1.37^{a}	1.47^{c}	1.69^{c}	1.56^{c,d}
SEM[1]	0.0020	0.0047	0.0073	0.0122	0.0011	0.0039	0.0069	0.0100	0.0073	0.0095	0.0124	0.009
HB (%)												
0	0.279	0.660^{a}	0.963	1.903	0.339	1.037	1.613^{a}	2.989	1.19	1.45	1.65	1.51
30	0.273	0.645^{a}	0.927	1.846	0.342	1.034	1.564^{b}	2.939	1.22	1.48	1.66	1.53
60	0.248	0.596^{b}	0.843	1.686	0.333	0.998	1.536^{b}	2.867	1.31	1.55	1.80	1.64
BGase (%)												
0	0.262	0.615^{b}	0.889	1.766	0.334	1.017	1.586^{a}	2.937	1.25	1.53	1.77	1.61
0.01	0.271	0.640^{a}	0.912	1.823	0.341	1.033	1.592^{a}	2.966	1.23	1.49	1.72	1.56
0.1	0.267	0.646^{a}	0.932	1.845	0.339	1.018	1.535^{b}	2.893	1.25	1.46	1.63	1.51
ANOVA *P*-value												
HB	<0.001	<0.001	<0.001	<0.001	0.002	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001
BGase	0.004	0.003	<0.001	<0.001	0.005	0.028	<0.001	<0.001	0.004	0.003	<0.001	<0.001
HB × BGase	<0.001	0.094	0.002	0.002	0.042	0.004	0.676	0.030	<0.001	0.015	<0.001	<0.001

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

Abbreviations: BGase, β-glucanase; HB, hulless barley.

[1]SEM = pooled standard error of mean (means of 9 replications).

Table 4. Effects of hulless barley and β-glucanase on mortality of broiler chickens from 0 to 32 d.

Item	Mortality (%)
Hulless barley (%) × β-glucanase (%)	
0 × 0	4.3
0 × 0.01	3.5
0 × 0.1	4.0
30 × 0	3.5
30 × 0.01	4.6
30 × 0.1	4.5
60 × 0	3.5
60 × 0.01	4.5
60 × 0.1	3.1
SEM[1]	0.304
Hulless barley (%)	
0	3.9
30	4.2
60	3.7
β-glucanase (%)	
0	3.7
0.01	4.2
0.1	3.9
ANOVA *P*-value	
Hulless barley	0.813
β-glucanase	0.823
Hulless barley × β-glucanase	0.724

[1]SEM = pooled standard error of mean (means of 9 replications).

for the birds fed 60% HB. From 11 to 22 d, BWG was lower for the 60% HB birds in comparison with those from the 0 and 30% HB treatments: both levels of BGase addition increased BWG for this period. Feed intake from 11 to 22 d was not affected by enzyme addition for the birds fed 0 and 30% HB levels, but 0.01% BGase resulted in higher FI than for the other 2 enzyme levels for the 60% HB diet. Feed to gain ratio was also not affected by enzyme level for the 2 lower HB levels, but the 0.1% BGase level resulted in a lower value than 0 or 0.01% BGase treatments at 60% HB diet inclusion. Enzyme addition again did not affect BWG from 22 to 32 d, when included in the diets containing 0 and 30% HB. However, broilers fed the 60% HB diets grew faster with enzyme supplementation. With the same period, FI was lower in the diets containing 30 and 60% HB than the wheat diet, and lower for the 0.1% BGase treatment than for the birds fed diets with 0 or 0.01% BGase supplementation. Optimum FCR during the 22–32 d period was achieved with 0.1% enzyme inclusion in the 0 and 30% HB diets. For the birds on the 30% HB diets, the 0.1% BGase treatment was superior to the unsupplemented diet. Finally, for 60% HB treatments, F:G improved as the level of BGase increased. Overall (0–32 d), enzyme did not affect BWG for 0 and 30% HB diets, but both enzyme levels resulted in more gain when the diets contain 60% HB. Feed intake increased with the 0.01% BGase in comparison with 0 and 0.01% BGase when the birds were fed 60% HB diets. However, there was no BGase effect on FI at 0 and 30% HB treatments. Feed-to-gain ratio was lower with 0.1% BGase than with 0% BGase at both 0 and 30% HB diets, but F:G decreased with increasing BGase level in the birds fed 60% HB diets.

The total mortality of the flock was 4.1% and not affected by HB or BGase (Table 4). Only 3.8% of the total mortality was confirmed as coccidiosis by necropsy. However, 43.3% of the total mortality was diagnosed as either necrotic enteritis or systemic infection, with the latter possibly due to the destruction of the intestinal epithelial membrane and bacterial translocation because of subclinical coccidiosis. These data support the conclusion that vaccination with Coccivac-B52 induced a disease challenge in experimental birds.

Table 5. Effects of hulless barley and β-glucanase on ileal digesta viscosity of broiler chickens.

	Viscosity (cP)	
Item	Day 11	Day 33
Hulless barley (%) × β-glucanase (%)		
0 × 0	7.84	2.37[b]
0 × 0.01	3.48	2.36[b]
0 × 0.1	3.37	2.83[a,b]
30 × 0	7.11	3.67[a,b]
30 × 0.01	6.93	3.38[a,b]
30 × 0.1	3.66	3.13[a,b]
60 × 0	9.73	3.98[a]
60 × 0.01	5.31	3.46[a,b]
60 × 0.1	3.53	2.30[b]
SEM[1]	0.431	0.120
Main effects		
Hulless barley (%)		
0	4.90	2.52
30	5.90	3.39
60	6.19	3.25
β-glucanase (%)		
0	8.23[a]	3.34
0.01	5.24[b]	3.07
0.1	3.52[b]	2.76
ANOVA *P*-value		
Hulless barley	0.261	0.002
β-glucanase	<0.001	0.086
Hulless barley × β-glucanase	0.160	0.021

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

[1]SEM = pooled standard error of mean (Day 11: means of 6 replications/Day 33: means of 9 replications; pooled ileal samples from 2 birds per replicate in each day).

Viscosity

The ileal digesta viscosity was only affected by BGase at 11 d with both 0.01 and 0.1% BGase, similarly, reducing viscosity (Table 5). The interaction between the main effects was significant for viscosity at day 33. In birds fed 0 and 30% HB, BGase did not affect viscosity, whereas in the 60% HB diets, the highest level of BGase decreased the viscosity compared with the 0% BGase inclusion level.

Gastrointestinal Tract Morphology

At day 11, relative GIT segment empty weights, lengths, organ weights, and content weights were affected by HB and BGase. Empty weights of the proventriculus, gizzard, jejunum, small intestine, and colon, and the lengths of the jejunum, small intestine, and colon increased with increasing levels of HB (Table 6). In all cases, 0 and 60% HB values were different, and values for 30% HB were either statistically intermediate or like either of the extremes. The 0% BGase level resulted in

Table 6. Effects of hulless barley and β-glucanase on gastrointestinal tissue weights and lengths (proportional to body weight) of broiler chickens aged 11 d.

	Empty weight (%)									Length (cm/100 g)					
Item	Crop	Proven	Gizz	Duo	Jej	Ileum	SI	Ceca	Colon	Duo	Jej	Ileum	SI	Ceca	Colon
HB (%) × BGase (%)															
0 × 0	0.44	0.73	2.37	1.56	2.56	1.83	5.97	0.56	0.22	6.35	15.09	14.09	35.30	5.06	1.31
0 × 0.01	0.52	0.77	2.27	1.66	2.54	1.84	6.03	0.57	0.21	6.27	14.84	14.14	35.24	4.95	1.14
0 × 0.1	0.43	0.70	2.24	1.69	2.45	1.71	5.85	0.50	0.18	6.15	13.83	13.25	33.22	4.54	1.15
30 × 0	0.49	0.87	2.40	1.73	2.59	1.88	6.19	0.54	0.22	6.20	14.88	13.74	34.81	4.73	1.21
30 × 0.01	0.47	0.81	2.44	1.63	2.55	1.77	5.95	0.54	0.22	6.37	14.87	14.08	35.32	4.66	1.21
30 × 0.1	0.44	0.73	2.31	1.69	2.61	1.89	6.17	0.58	0.21	6.33	14.92	14.32	35.57	4.83	1.27
60 × 0	0.49	0.85	2.66	1.72	2.69	1.93	7.00	0.58	0.25	7.17	16.43	14.61	40.29	5.01	1.39
60 × 0.01	0.49	0.77	2.43	1.68	2.81	1.90	6.38	0.58	0.24	6.40	15.76	14.55	36.70	5.07	1.23
60 × 0.1	0.48	0.78	2.58	1.73	2.65	1.83	6.27	0.61	0.24	6.50	14.90	14.36	37.02	5.12	1.35
SEM[1]	0.010	0.011	0.027	0.019	0.028	0.023	0.190	0.010	0.004	0.092	0.168	0.173	0.430	0.066	0.020
Main effects															
HB (%)															
0	0.46	0.73[b]	2.29[b]	1.64	2.52[b]	1.79	5.95[b]	0.55	0.20[b]	6.26	14.59[b]	13.82	34.58[b]	4.85	1.20[b]
30	0.47	0.80[a]	2.38[b]	1.68	2.58[a,b]	1.85	6.10[b]	0.55	0.22[b]	6.30	14.89[a,b]	14.04	35.23[b]	4.74	1.23[a,b]
60	0.49	0.80[a]	2.56[a]	1.71	2.71[a]	1.89	6.55[a]	0.59	0.24[a]	6.69	15.70[a]	14.50	38.00[a]	5.07	1.32[a]
BGase (%)															
0	0.47	0.81[a]	2.48	1.67	2.61	1.88	6.39	0.56	0.23[a]	6.57	15.47	14.15	36.80	4.93	1.30[a]
0.01	0.49	0.78[a,b]	2.38	1.66	2.63	1.83	6.12	0.57	0.22[a,b]	6.35	15.16	14.25	35.75	4.90	1.19[b]
0.1	0.45	0.74[b]	2.38	1.70	2.57	1.81	6.10	0.56	0.21[b]	6.33	14.55	13.98	35.27	4.83	1.25[a,b]
ANOVA *P*-value															
HB	0.421	0.014	<0.001	0.286	0.006	0.234	<0.001	0.097	<0.001	0.078	0.012	0.252	0.001	0.112	0.007
BGase	0.135	0.009	0.156	0.673	0.637	0.467	0.055	0.987	0.012	0.429	0.059	0.829	0.269	0.781	0.050
HB × BGase	0.235	0.214	0.362	0.438	0.679	0.585	0.103	0.276	0.656	0.389	0.554	0.774	0.267	0.380	0.278

[a-b]Means within a main effect or interaction not sharing a common superscript are significantly different ($P \leq 0.05$).
Abbreviations: BGase, β-glucanase; Duo, duodenum; Gizz, gizzard; HB, hulless barley; Jej, jejunum; Proven, proventriculus; SI, small intestine.
[1]SEM = pooled standard error of mean (n = 12 birds per treatment).

heavier proventriculus and colon weights, and colon length in comparison with either or both 0.01 and 0.1% enzyme levels.

The 60% HB treatments resulted in more digesta content in the crop, gizzard, jejunum, and colon than the 0% HB treatment; digesta content for the birds from the 30% HB diets were either intermediate or statistically equal to 0 or 60% values. Interactions between HB and BGase treatments were found for the digesta content of the crop, duodenum, ileum, and ceca. The crop, duodenum, and cecal interactions did not follow apparent trends (Table 7). The interaction for the ileum and small intestine revealed some differences in the pattern of response to treatments, but the main effects predominated with more digesta content for 60% than 30 and 0% HB treatments, and more digesta content for the 0% than the 0.01 and 0.1% BGase treatments. An interaction was also found for liver weight with the enzyme not affecting 0 and 30% HB treatments, but the 0.1% BGase treatment resulting in heavier weights than 0% BGase for 60% HB diets; the 0.01% BGase treatment was intermediate and not statistically different than the other enzyme treatments. The spleen weights were proportionally heavier for 0 and 0.01% BGase than the 0.1% BGase treatment. The pancreas weight increased with increasing levels of HB, and the weight for the 0.1% enzyme addition was lower than for the 0.01% BGase treatment with the 0% BGase treatment being intermediate.

Gastrointestinal tract size and content weights were affected by HB and BGase at day 33. As seen at 11 d, the digestive tract empty weights (gizzard, duodenum, jejunum, small intestine, ceca, and colon) and lengths (duodenum, small intestine, ceca, and colon) increased with the addition of HB (Table 8). The 0 and 60% HB treatments were consistently different, and the 30% HB values were either intermediate or more closely aligned with either the 0 or 60% HB inclusion levels. Interactions between the main effects were seen for the jejunum and ileum lengths. In both cases, the BGase level did not affect the lengths for the birds consuming 0 and 30% HB, but enzyme addition (0.01 and 0.1%) reduced the lengths when 60% HB was fed.

The digesta content weights of the crop, gizzard, ileum, ceca, and colon were highest for the 60% HB diets, and except for the crop, lowest for the 0% HB diets (Table 9). The addition of 0.1% BGase reduced the content weight in the jejunum, ileum, and colon compared with not adding an enzyme to the diet. An interaction was found for the content weight of the small intestine, which was lower with 0.1% than with 0% BGase for the birds fed 60% HB, but the differences between these 2 diets did not approach significance for the 30 or 0% HB diets. The liver and pancreas weights increased with the level of diet HB, whereas the liver weights were lower when BGase was included in the diet.

DISCUSSION

Performance variables of broiler chickens in this study were within a normal range in accordance with Ross 308 Broiler Performance Objectives (Aviagen, 2014), and were affected by treatment in an age-dependent manner. Overall, performance variables decreased with the

Table 7. Effects of hulless barley and β-glucanase on gastrointestinal content and organ weights as a percentage of body weight of broiler chickens aged 11 d.

	Content (%)									Weight (%)		
Item	Crop	Proven	Gizz	Duo	Jej	Ileum	SI	Ceca	Colon	Liver	Spleen	Pancreas
HB (%) × BGase (%)												
0 × 0	0.23^{c}	0.06	0.57	0.05^{a,b}	0.44	0.36^{b,c}	0.84^{b,c}	0.11^{a,b}	0.03	4.67^{a}	0.13	0.44
0 × 0.01	0.44^{a,b,c}	0.08	0.67	0.04^{b}	0.44	0.40^{b,c}	0.87^{b,c}	0.14^{a}	0.03	4.47^{a,b}	0.12	0.47
0 × 0.1	0.43^{a,b,c}	0.05	0.59	0.04^{b}	0.40	0.28^{c}	0.71^{c}	0.06^{b}	0.02	4.46^{a,b}	0.11	0.40
30 × 0	0.43^{a,b,c}	0.17	0.80	0.04^{b}	0.46	0.38^{b,c}	0.88^{b,c}	0.09^{a,b}	0.04	4.29^{a,b}	0.12	0.45
30 × 0.01	0.29^{a,b,c}	0.11	0.80	0.04^{b}	0.39	0.32^{b,c}	0.74^{b,c}	0.06^{b}	0.04	4.41^{a,b}	0.13	0.50
30 × 0.1	0.28^{b,c}	0.06	0.72	0.05^{a,b}	0.41	0.36^{b,c}	0.80^{b,c}	0.09^{a,b}	0.04	4.43^{a,b}	0.10	0.46
60 × 0	0.48^{a,b,c}	0.06	0.89	0.08^{a}	0.59	0.60^{a}	1.26^{a}	0.08^{a,b}	0.06	4.12^{b}	0.13	0.54
60 × 0.01	0.50^{a,b}	0.05	0.75	0.03^{b}	0.49	0.43^{b}	0.95^{b}	0.06^{b}	0.06	4.49^{a,b}	0.13	0.50
60 × 0.1	0.54^{a}	0.05	0.81	0.05^{a,b}	0.45	0.41^{b,c}	0.89^{b,c}	0.12^{a,b}	0.04	4.61^{a}	0.11	0.50
SEM[1]	0.023	0.010	0.022	0.003	0.012	0.013	0.022	0.006	0.002	0.039	0.002	0.006
Main effects												
HB (%)												
0	0.37	0.06^{a,b}	0.61^{b}	0.04	0.43^{b}	0.34	0.81	0.10	0.03^{b}	4.53	0.12	0.44^{c}
30	0.33	0.11^{a}	0.77^{a}	0.04	0.42^{b}	0.35	0.81	0.08	0.04^{b}	4.38	0.12	0.47^{b}
60	0.51	0.05^{b}	0.82^{a}	0.05	0.51^{a}	0.48	1.03	0.08	0.05^{a}	4.41	0.13	0.51^{a}
BGase (%)												
0	0.38	0.09	0.75	0.05	0.50^{a}	0.45	0.99	0.09	0.04^{a}	4.36	0.12^{a}	0.48^{a,b}
0.01	0.41	0.08	0.74	0.03	0.44^{a,b}	0.38	0.86	0.08	0.04^{a}	4.45	0.13^{a}	0.49^{a}
0.1	0.42	0.05	0.71	0.04	0.42^{b}	0.35	0.80	0.09	0.03^{b}	4.50	0.11^{b}	0.45^{b}
ANOVA *P*-value												
HB	0.001	0.029	0.002	0.090	0.002	<0.001	<0.001	0.178	0.001	0.184	0.204	<0.001
BGase	0.727	0.225	0.641	0.033	0.013	0.004	<0.001	0.758	0.022	0.232	0.002	0.017
HB × BGase	0.016	0.323	0.359	0.035	0.400	0.003	0.006	0.001	0.436	0.003	0.776	0.116

[a-c]Means within a main effect or interaction not sharing a common superscript are significantly different ($P \leq 0.05$).
Abbreviations: BGase, β-glucanase; Duo, duodenum; Gizz, gizzard; HB, hulless barley; Jej, jejunum; Proven, proventriculus; SI, small intestine.
[1]SEM = pooled standard error of mean (n = 12 birds per treatment).

substitution of wheat with HB in the broiler diets, which is at least partially associated with the high level of fiber and reduced energy and starch content in HB compared with wheat (Coates et al., 1977; Bach Knudsen, 1997). The analyzed TDF was 26.7 and 14.4%, whereas total starch content was 53.7 and 62.8% in HB and wheat, respectively, in the present study, which supports this rationale. Therefore, the assumption which was made

Table 8. Effects of hulless barley and β-glucanase on gastrointestinal tissue weights and lengths (proportional to body weight) of broiler chickens aged 33 d.

	Empty weight (%)									Length (cm/100 g)					
Item	Crop	Proven	Gizz	Duo	Jej	Ileum	SI	Ceca	Colon	Duo	Jej	Ileum	SI	Ceca	Colon
HB (%) × BGase (%)															
0 × 0	0.28	0.37	0.97	0.78	1.48	0.96	3.21	0.31	0.12	1.44	3.53^{b,c}	3.46^{b}	8.42	1.35	0.31
0 × 0.01	0.28	0.35	1.01	0.82	1.49	1.00	3.30	0.33	0.13	1.52	3.71^{b,c}	3.49^{b}	8.71	1.34	0.34
0 × 0.1	0.29	0.36	1.03	0.80	1.44	1.05	3.28	0.34	0.12	1.48	3.28^{c}	3.38^{b}	8.14	1.40	0.32
30 × 0	0.28	0.40	1.04	0.84	1.48	1.05	3.37	0.36	0.15	1.63	3.83^{b,c}	3.86^{b}	9.31	1.60	0.35
30 × 0.01	0.27	0.42	1.11	0.85	1.48	1.03	3.36	0.35	0.14	1.54	3.55^{b,c}	3.42^{b}	8.50	1.43	0.34
30 × 0.1	0.29	0.37	1.10	0.82	1.46	0.99	3.27	0.37	0.13	1.55	3.70^{b,c}	3.76^{b}	9.00	1.43	0.35
60 × 0	0.30	0.38	1.12	0.87	1.64	1.13	3.64	0.37	0.17	1.80	4.49^{a}	4.42^{a}	10.70	1.69	0.41
60 × 0.01	0.29	0.39	1.18	0.85	1.59	1.09	3.52	0.37	0.17	1.64	3.89^{b}	3.87^{b}	9.39	1.53	0.40
60 × 0.1	0.29	0.39	1.23	0.86	1.53	1.00	3.38	0.38	0.15	1.63	3.88^{b}	3.86^{b}	9.37	1.53	0.38
SEM[1]	0.004	0.008	0.017	0.010	0.016	0.015	0.031	0.005	0.003	0.022	0.047	0.048	0.101	0.030	0.006
Main effects															
HB (%)															
0	0.28	0.36	1.00^{b}	0.80^{b}	1.47^{b}	1.00	3.26^{b}	0.33^{b}	0.12^{c}	1.48^{b}	3.51	3.44	8.42^{c}	1.37^{b}	0.32^{b}
30	0.28	0.39	1.08^{b}	0.84^{a,b}	1.47^{b}	1.02	3.33^{b}	0.36^{a}	0.14^{b}	1.57^{a,b}	3.69	3.68	8.94^{b}	1.49^{a,b}	0.35^{b}
60	0.29	0.39	1.18^{a}	0.86^{a}	1.59^{a}	1.07	3.51^{a}	0.37^{a}	0.17^{a}	1.69^{a}	4.09	4.05	9.82^{a}	1.58^{a}	0.40^{a}
BGase (%)															
0	0.29	0.38	1.04	0.83	1.53	1.05	3.41	0.35	0.15^{a}	1.62	3.95	3.91	9.48^{a}	1.55	0.36
0.01	0.28	0.38	1.10	0.84	1.52	1.04	3.39	0.35	0.14^{a,b}	1.56	3.72	3.59	8.87^{b}	1.44	0.36
0.1	0.29	0.37	1.12	0.83	1.48	1.01	3.31	0.37	0.13^{b}	1.55	3.62	3.67	8.83^{b}	1.55	0.35
ANOVA *P*-value															
HB	0.498	0.136	0.001	0.028	0.002	0.139	0.002	0.001	0.001	0.003	0.001	0.001	<0.001	0.007	0.001
BGase	0.477	0.670	0.130	0.880	0.272	0.589	0.390	0.168	0.017	0.333	0.004	0.004	0.002	0.206	0.870
HB × BGase	0.757	0.619	0.967	0.835	0.849	0.196	0.488	0.972	0.122	0.338	0.012	0.047	0.010	0.658	0.383

[a-c]Means within a main effect or interaction not sharing a common superscript are significantly different ($P \leq 0.05$).
Abbreviations: BGase, β-glucanase; Duo, duodenum; Gizz, gizzard; HB, hulless barley; Jej, jejunum; Proven, proventriculus; SI, small intestine.
[1]SEM = pooled standard error of mean (n = 18 birds per treatment).

Table 9. Effects of hulless barley and β-glucanase on gastrointestinal content and organ weights as a percentage of body weight of broiler chickens aged 33 d.

	Content (%)									Weight (%)		
Item	Crop	Proven	Gizz	Duo	Jej	Ileum	SI	Ceca	Colon	Liver	Spleen	Pancreas
HB (%) × BGase (%)												
0 × 0	1.15	0.18	0.75	0.13	1.04	0.93	2.09^{b,c}	0.22	0.12	2.83	0.11	0.23
0 × 0.01	1.36	0.08	0.82	0.12	1.04	0.89	2.05^{b,c}	0.21	0.15	2.88	0.12	0.25
0 × 0.1	1.19	0.10	0.85	0.13	0.92	0.69	1.73^{c}	0.20	0.09	2.80	0.12	0.23
30 × 0	1.10	0.19	0.97	0.12	1.12	1.12	2.36^{a,b,c}	0.26	0.17	3.15	0.14	0.26
30 × 0.01	0.85	0.22	1.05	0.11	1.02	0.96	2.08^{b,c}	0.22	0.14	2.89	0.12	0.25
30 × 0.1	1.08	0.06	1.05	0.14	1.01	1.04	2.19^{b,c}	0.23	0.14	2.87	0.12	0.27
60 × 0	1.54	0.11	1.18	0.12	1.31	1.49	2.91^{a}	0.27	0.23	3.16	0.12	0.27
60 × 0.01	1.29	0.07	1.35	0.09	1.19	1.28	2.55^{a,b}	0.22	0.18	2.90	0.13	0.28
60 × 0.1	1.44	0.06	1.33	0.09	0.86	0.97	1.91^{b,c}	0.32	0.14	2.88	0.12	0.27
SEM[1]	0.064	0.018	0.036	0.005	0.027	0.034	0.056	0.010	0.007	0.026	0.003	0.004
Main effects												
HB (%)												
0	1.23^{a,b}	0.12	0.81^{c}	0.13	1.00	0.84^{c}	1.96	0.21^{b}	0.12^{b}	2.83^{b}	0.12	0.24^{b}
30	1.01^{b}	0.15	1.03^{b}	0.12	1.05	1.04^{b}	2.21	0.24^{a,b}	0.15^{a,b}	2.97^{a,b}	0.12	0.26^{a,b}
60	1.42^{a}	0.08	1.29^{a}	0.10	1.12	1.24^{a}	2.46	0.27^{a}	0.18^{a}	2.98^{a}	0.13	0.27^{a}
BGase (%)												
0	1.26	0.16	0.97	0.12	1.16^{a}	1.18^{a}	2.45	0.25	0.17^{a}	3.04^{a}	0.12	0.25
0.01	1.17	0.12	1.07	0.11	1.08^{a}	1.04^{a,b}	2.22	0.22	0.16^{a,b}	2.89^{b}	0.12	0.26
0.1	1.23	0.07	1.08	0.12	0.93^{b}	0.90^{b}	1.94	0.25	0.13^{b}	2.85^{b}	0.12	0.26
ANOVA *P*-value												
HB	0.035	0.261	<0.001	0.075	0.176	<0.001	0.001	0.039	0.001	0.031	0.599	0.002
BGase	0.826	0.156	0.306	0.364	0.002	0.001	0.004	0.275	0.015	0.004	0.956	0.448
HB × BGase	0.697	0.518	0.987	0.455	0.116	0.090	0.041	0.284	0.141	0.152	0.274	0.431

[a-c]Means within a main effect or interaction not sharing a common superscript are significantly different ($P \leq 0.05$).
Abbreviations: BGase, β-glucanase; Duo, duodenum; Gizz, gizzard; HB, hulless barley; Jej, jejunum; Proven, proventriculus; SI, small intestine.
[1]SEM = pooled standard error of mean (n = 18 birds per treatment).

during the diet formulation regarding the approximately similar nutrient content of HB and wheat was not accurate. Consequently, the broiler performance was reduced with the addition of HB to the wheat-based diets. In addition, comparatively lower nutrient digestibility might also be related to the poor performance in the broilers fed HB-based diets in comparison with wheat. The ileal viscosity was higher with the 60 than 0% HB in broilers given the diets with 0% BGase at day 11 and to a lesser extent day 33. Therefore, the increased ileal digesta viscosity might affect the digestibility of nutrients, including fat, starch, and protein (Edney et al., 1989; Rodríguez et al., 2012), and in turn affect broiler performance (Jacob and Pescatore, 2014). Furthermore, high fiber levels in the HB cell wall might encapsulate nutrients, including starch and protein, and thereby reduce access to digestive enzymes and affect nutrient digestibility in chickens (Hesselman and Åman, 1986). The lower digestibility of HB than that of wheat-based diets was also supported by the higher digestive tract tissue weights (including pancreas weight) and lengths, and digesta content reported in the current research because the GIT attempts to compensate for the nutrient digestion process by increasing GIT size and feed retention time (Salih et al., 1991; Brenes et al., 1993; Jørgensen et al., 1996).

Body weight gain and F:G improved with the 0.01% level of BGase, whereas there was a reduction or no improvement with the 0.1% level of the enzyme when broilers were fed 60% HB from day 0 to 11. By contrast, production performance increased with both 0.01 and 0.1% dosages of BGase, and the 0.1% BGase resulted in better performance than 0.01% BGase in broilers after day 11. The differences in BGase dose effects on performance do not appear to relate to digesta viscosity because, at both ages, viscosity was numerically lower for the 0.1% than for the 0.01% BGase level. Furthermore, the only significant effect of BGase on viscosity was observed for the 60% HB diet at day 33, yet improvements in F:G were found for all levels of HB. Therefore, digesta viscosity cannot be identified as the primary mechanism of action that affects broiler performance. This conclusion is further supported by the absence of HB effect on viscosity at day 11.

The variation of BGase level effects on the broiler performance at different ages may indicate that young and older broilers vary in their ability to effectively utilize lower MW, fermentable carbohydrates. The levels of SCFA in the ceca increased with 0.1% compared with 0.01% BGase level, while reducing cecal pH (with increasing BGase levels) in these young broilers fed 60% HB diets (N. D. Karunaratne, unpublished data), suggesting fermentation is possible; however, SCFA levels might not be adequate to considerably increase the bird performance (Ribeiro et al., 2018). Furthermore, the observed increase in low MW, indigestible β-glucan load with the highest BGase treatment (Karunaratne and Classen, 2019) might cause an undesirable effect on the gut microbiota in the broilers, particularly in the diseased state induced by coccidiosis challenge at 5 d of age. Furthermore, microbial population changes associated with an increased indigestible carbohydrate load in young broiler chickens with a less mature digestive tract and a less diversified microbial

population (Lu et al., 2003; Awad et al., 2016; Ocejo et al., 2019) may have negatively affected performance. Therefore, it might further reduce the performance, although the particular bacteria involved in this gut microbial shift is unknown in the present study. By contrast, maturation of the digestive tract with age (Dibner et al., 1996; Iji et al., 2001) and establishment of a beneficial and diverse gastrointestinal microbiota capable of effectively utilizing the lower MW carbohydrates from depolymerization of high MW glucan (Knarreborg et al., 2002; van der Wielen et al., 2002; Lu et al., 2003) may be responsible for the production improvement in older birds. It has recently been demonstrated that the ability of the cecal microbiome to digest soluble xylan increases with age and can be accelerated by the use of xylanase (Bautil et al., 2019). We suggest that a similar adaptation and BGase stimulation of digestive tract microbiota to digest β-glucan with age is also likely the case, and excessive stimulation early in the bird's life may be detrimental in terms of performance.

The age effect on viscosity is substantial in the present study, although the statistical separation of means is not possible. The ileal viscosity was lower at an older age, which agrees with previous research (Salih et al., 1991; Petersen et al., 1999; Lee et al., 2017). The age-related digesta viscosity reduction can also be explained by the increased ability of gut microbiota to secrete BGase. This, in turn, may result in a further adaptation of gut microbiota to utilize soluble β-glucan, which is in agreement with the effect of exogenous BGase on reducing the ileal digesta soluble β-glucan MW with the increasing age of broiler chickens given HB-based diets (Karunaratne and Classen, 2019). Furthermore, the increased utilization of soluble β-glucan with age is in accordance with the age-related adaptation of broiler gut microbiota for the water-extractable wheat arabinoxylan, which is indicated in Bautil et al. (2019). Hulless barley resulted in a higher viscosity compared with wheat, which is not unexpected because HB contains a comparatively higher quantity of high MW β-glucan (Biliaderis and Izydorczyk, 2007), and BGase reduced the viscosity because of the depolymerization of high MW β-glucan at day 33. By contrast, HB did not affect the ileal viscosity values at day 11, although BGase reduced the viscosity in these young birds. The disease status might affect the digesta viscosity because the birds were challenged for coccidiosis at day 5, and *Eimeria* may cause osmotic and absorptive changes in the host GIT, that reduce digesta viscosity (Crompton et al., 1976; Waldenstedt et al., 2000). Therefore, the effect of BGase might be subdued more at day 11 than at day 33 when the birds were assumed to be recovered. However, BGase might play a role in mitigating this disease condition by further reducing ileal viscosity and enabling more efficient digestion because the enzyme effect is significant on the viscosity, although it was lowered due to coccidiosis.

The improved broiler performance with increasing BGase levels might be associated with increased digestibility of nutrients. The relative empty weights and lengths of some digestive tract tissues decreased with BGase at day 11 and 33, and this might be associated with increased nutrient digestibility that leads to less requirement for a more extensive digestive tract. Beta-glucanase improves enzymatic efficiency of nutrient digestion, hence fewer enzymes are needed; consequently the pancreas is smaller. The higher digestibility might be related to the reduction of digesta viscosity or nutrient encapsulation by the endosperm cell wall (Hesselman and Åman, 1986; Masey-O'Neill et al., 2014). In addition, the decreased relative content weights of the GIT with increasing BGase level might be due to BGase-mediated lower feed retention time, which is associated with the reduction of digesta viscosity (Salih et al., 1991; Almirall and Esteve-Garcia, 1994).

Cereal β-glucan might contribute to increase broiler performance by modulating the immune system because oat β-glucan has been shown to positively affect immune function and digestive tract health in mice (Estrada et al., 1997; Yun et al., 1997, 2003). Furthermore, oat β-glucan increased the activation of dectin-1 receptors of human dendritic cells, and immune capacity when the β-glucan was pretreated with endoglucanase (Sahasrabudhe et al., 2016). It is suggested that endoglucanase treatment reduced β-glucan particle size and increased the surface area for specific enzyme-binding sites, which emphasizes microbial enzyme-mediated β-glucan depolymerization in the digestive tract. However, research has not been conducted regarding the effect of cereal β-glucan on immune function in chickens.

It is concluded that HB reduced the growth performance, whereas exogenous BGase increased the performance in a dose-dependent manner in broiler chickens. However, the highest level of exogenous BGase did not affect or, in the case of feed to gain ratio, reduced the performance of 0–11 d old broilers fed 60% HB-based diets suggesting a period of time early on in the broilers life where excess BGase can be detrimental. Overall, HB increased the digestive tract tissue weights and lengths, and digesta content, whereas exogenous BGase decreased the same parameters.

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REFERENCES

AACC International. 2010. Approved methods of analysis. 11th ed. American Association of Cereal Chemists, St. Paul, MN.

Almirall, M., and E. Esteve-Garcia. 1994. Rate of passage of barley diets with chromium oxide: influence of age and poultry strain and effect of beta-glucanase supplementation. Poult. Sci. 73:1433–1440.

AOAC. 2006. Official Methods of Analysis. 18th. ed. Association of Official Analytical Chemists, Arlington, VA.

Aviagen. 2014. Ross 308 Broiler performance objectives. Accessed Oct. 2017. https://www.winmixsoft.com/files/info/Ross-308-Broiler-PO-2014-EN.pdf.

Awad, W. A., E. Mann, M. Dzieciol, C. Hess, S. Schmitz-Esser, M. Wagner, and M. Hess. 2016. Age-related differences in the luminal and mucosa-associated gut microbiome of broiler chickens and shifts associated with *Campylobacter jejuni* infection. Front. Cell. Infect. Microbiol. 6:154.

Bach Knudsen, K. E. 1997. Carbohydrate and lignin contents of plant materials used in animal feeding. Anim. Feed Sci. Technol. 67:319–338.

Bautil, A., J. Verspreet, J. Buyse, P. Goos, M. R. Bedford, and C. M. Courtin. 2019. Age-related arabinoxylan hydrolysis and fermentation in the gastrointestinal tract of broiler fed wheat-based diets. Poult. Sci. 98:4606–4621.

Bedford, M. R., and K. Autio. 1996. Microscopic examination of feed and digesta from wheat-fed broiler chickens and its relation to bird performance. Poult. Sci. 75(Supp1):1 (Abstr.).

Bedford, M. R. 2019. Chapter 21: Future prospects for non-starch polysaccharide degrading enzymes development in monogastric nutrition. Pages 373–383 in The Value of Fibre. Wageningen Academic Publishers, Wageningen, the Netherlands.

Biliaderis, C. G., and M. S. Izydorczyk. 2007. Page 570 in Functional Food Carbohydrates. CRC Press, London, NY.

Brenes, A., M. Smith, W. Guenter, and R. R. Marquardt. 1993. Effect of enzyme supplementation on the performance and digestive tract size of broiler chickens fed wheat- and barley-based diets. Poult. Sci. 72:1731–1739.

Canadian Council on Animal Care. 1993. Guide to the Care and Use of Experimental Animals, 1. E. D. Olfert, B. M. Cross, and A. A. McWilliam, eds. 2nd ed. Canadian Council on Animal Care, Ottawa, ON, Canada.

Canadian Council on Animal Care in science. 2009. CCAC Guidelines on: The Care and Use of Farm Animals in Research, Teaching and Testing. Canadian Council on Animal Care, Ottawa, ON, Canada.

Campbell, G. L., B. G. Rossnagel, H. L. Classen, and P. A. Thacker. 1989. Genotypic and environmental differences in extract viscosity of barley and their relationship to its nutritive value for broiler chickens. Anim. Feed Sci. Technol. 26:221–230.

Choct, M., A. Kocher, D. L. E. Waters, D. Pettersson, and G. Ross. 2004. A comparison of three xylanases on the nutritive value of two wheats for broiler chickens. Br. J. Nutr. 92:53–61.

Classen, H. L., G. L. Campbell, and J. W. D. Grootwassink. 1988. Improved feeding value of Saskatchewan-grown barley for broiler chickens with dietary enzyme supplementation. Can. J. Anim. Sci. 68:1253–1259.

Coates, B. J., S. J. Slinger, J. D. Summers, and H. S. Bayley. 1977. Metabolizable energy values and chemical and physical characteristics of wheat and barley. Can. J. Anim. Sci. 57:195–207.

Crompton, D. W. T. 1976. Page 311 in Digestion in the Fowl. British Poultry Science Ltd., Edinburgh, UK.

De Maesschalck, C., V. Eeckhaut, L. Maertens, L. D. Lange, L. Marchal, C. Nezer, S. D. Baere, S. Croubels, G. Daube, J. Dewulf, F. Haesebrouck, R. Ducatelle, B. Taminau, and F. V. Immerseel. 2015. Effects of xylo-oligosaccharides on broiler chicken performance and microbiota. Appl. Environ. Microbiol. 81:5880–5888.

Dibner, J. J., M. L. Kitchell, C. A. Atwell, and F. J. Ivey. 1996. The effect of dietary ingredients and age on the microscopic structure of the gastrointestinal tract in poultry1. J. Appl. Poult. Res. 5:70–77.

Dos Santos, T. T., R. A. H. M Ten Doeschate, K. van de Belt, and J. D van der Klis. 2013. Evaluation of the additive effects of xylanase and glucanase in wheat, barley and wheat/barley based broiler diets. Proc. 19th Eur. Sym. Poult. Nutr., Potsdam, Germany. August 2013:26-29.

Ducatelle, R., V. Eeckhaut, F. Haesebrouck, and F. Van Immerseel. 2015. A review on prebiotics and probiotics for the control of dysbiosis: present status and future perspectives. Animal 9:43–48.

Edney, M. J., G. L. Campbell, and H. L. Classen. 1989. The effect of β-glucanase supplementation on nutrient digestibility and growth in broilers given diets containing barley, oat groats or wheat. Anim. Feed Sci. Technol. 25:193–200.

Estrada, A., C. H. Yun, A. Van Kessel, B. Li, S. Hauta, and B. Laarveld. 1997. Immunomodulatory activities of oat beta-glucan in vitro and in vivo. Microbiol. Immunol. 41:991–998.

Fuente, J. M., P. Pérez de Ayala, and M. J. Villamide. 1995. Effect of dietary enzyme on the metabolizable energy of diets with increasing levels of barley fed to broilers at different ages. Anim. Feed Sci. Technol. 56:45–53.

Gadde, U., W. H. Kim, S. T. Oh, and H. S. Lillehoj. 2017. Alternatives to antibiotics for maximizing growth performance and feed efficiency in poultry: a review. Anim. Health Res. Rev. 18:26–45.

Hesselman, K., and P. Åman. 1986. The effect of β-glucanase on the utilization of starch and nitrogen by broiler chickens fed on barley of low- or high-viscosity. Anim. Feed Sci. Technol. 15:83–93.

ICC. 2011. ICC Standard Methods. International Association for Cereal Science and Technology, Vienna, Austria.

Iji, P. A., A. Saki, and D. R. Tivey. 2001. Body and intestinal growth of broiler chicks on a commercial starter diet. 1. Intestinal weight and mucosal development. Br. Poult. Sci. 42:505–513.

Jacob, J. P., and A. J. Pescatore. 2014. Barley β-glucan in poultry diets. Ann. Transl. Med. 2:20–26.

Jørgensen, H., X. Q. Zhao, K. E. Knudsen, and B. O. Eggum. 1996. The influence of dietary fibre source and level on the development of the gastrointestinal tract, digestibility and energy metabolism in broiler chickens. Br. J. Nutr. 75:379–395.

Józefiak, D., S. Kaczmarek, A. Rutkowski, A. Józefiak, B. B. Jensen, and R. M. Engberg. 2005. Fermentation in broiler chicken gastrointestinal tract as affected by high dietary inclusion of barley and by β-glucanase supplementation. J. Anim. Feed Sci. 14:695–704.

Józefiak, D., A. Rutkowski, B. B. Jensen, and R. M. Engberg. 2006. The effect of β-glucanase supplementation of barley- and oat-based diets on growth performance and fermentation in broiler chicken gastrointestinal tract. Br. Poult. Sci. 47:57–64.

Karunaratne, N. D., and H. L. Classen. 2019. Chapter 10: Beta-glucans and beta-glucanase in animal nutrition, do we understand their full effects?. Pages 171–191 in The Value of Fibre. Wageningen Academic Publishers, Wageningen, the Netherlands.

Knarreborg, A., M. A. Simon, R. M. Engberg, B. B. Jensen, and G. W. Tannock. 2002. Effects of dietary fat source and subtherapeutic levels of antibiotic on the bacterial community in the ileum of broiler chickens at various ages. Appl. Environ. Microbiol. 68:5918–5924.

Lee, S. A., J. Apajalahti, K. Vienola, G. González-Ortiz, C. M. G. A. Fontes, and M. R. Bedford. 2017. Age and dietary xylanase supplementation affects ileal sugar residues and short chain fatty acid concentration in the ileum and caecum of broiler chickens. Anim. Feed Sci. Technol. 234:29–42.

Lu, J., U. Idris, B. Harmon, C. Hofacre, J. J. Maurer, and M. D. Lee. 2003. Diversity and succession of the intestinal bacterial community of the maturing broiler chicken. Appl. Environ. Microbiol. 69:6816–6824.

Masey-O'neill, H. V., M. Singh, and A. J. Cowieson. 2014. Effects of exogenous xylanase on performance, nutrient digestibility, volatile

fatty acid production and digestive tract thermal profiles of broilers fed on wheat- or maize-based diet. Br. Poult. Sci. 55:351–359.

Morgan, N. K., A. Wallace, M. R. Bedford, and M. Choct. 2017. Efficiency of xylanases from families 10 and 11 in production of xylo-oligosaccharides from wheat arabinoxylans. Carbohyd. Polym. 167:290–296.

Ocejo, M., B. Oporto, and A. Hurtado. 2019. 16S rRNA amplicon sequencing characterization of caecal microbiome composition of broilers and free-range slow-growing chickens throughout their productive lifespan. Sci. Rep. 9:1–14.

Perttilä, S., J. Valaja, K. Partanen, T. Jalava, T. Kiiskinen, and S. Palander. 2001. Effects of preservation method and β-glucanase supplementation on ileal amino acid digestibility and feeding value of barley for poultry. Br. Poult. Sci. 42:218–229.

Petersen, S. T., J. Wiseman, and M. R. Bedford. 1999. Effects of age and diet on the viscosity of intestinal contents in broiler chicks. Br. Poult. Sci. 40:364–370.

Potter, L. M., M. W. Stutz, and L. D. Matterson. 1965. Metabolizable energy and digestibility coefficients of barley for chicks as influenced by water treatment or by presence of fungal enzyme. Poult. Sci. 44:565–573.

Ravn, J. L., V. Glitsø, D. Pettersson, R. Ducatelle, F. Van Immerseel, and N. R. Pedersen. 2018. Combined endo-β-1,4-xylanase and α-L-arabinofuranosidase increases butyrate concentration during broiler cecal fermentation of maize glucurono-arabinoxylan. Anim. Feed Sci Technol. 236:159–169.

Ribeiro, T., V. Cardoso, L. M. A. Ferreira, M. M. S. Lordelo, E. Coelho, A. S. P. Moreira, M. R. M. Domingues, M. A. Coimbra, M. R. Bedford, and C. M. G. A. Fontes. 2018. Xylo-oligosaccharides display a prebiotic activity when used to supplement wheat or corn-based diets for broilers. Poult. Sci. 97:4330–4341.

Rodríguez, M. L., A. Rebolé, S. Velasco, L. T. Ortiz, J. Treviño, and C. Alzueta. 2012. Wheat- and barley-based diets with or without additives influence broiler chicken performance, nutrient digestibility and intestinal microflora. J. Sci. Food Agric. 92:184–190.

Sahasrabudhe, N. M., L. Tian, M. van den Berg, G. Bruggeman, E. Bruininx, H. A. Schols, M. M. Faas, and P. de Vos. 2016. Endo-glucanase digestion of oat beta-Glucan enhances Dectin-1 activation in human dendritic cells. J. Func. Foods 21:104–112.

Salih, M. E., H. L. Classen, and G. L. Campbell. 1991. Response of chickens fed on hull-less barley to dietary β-glucanase at different ages. Anim. Feed Sci. Technol. 33:139–149.

SAS Institute. 2008. SAS User's Guide: Statistics. Version 9. 4th ed. SAS Institute. Inc., Cary, NC.

van der Wielen, P. W. J. J., D. A. Keuzenkamp, L. J. A. Lipman, F. van Knapen, and S. Biesterveld. 2002. Spatial and temporal variation of the intestinal bacterial community in commercially raised broiler chickens during growth. Microb. Ecol. 44:286–293.

Waldenstedt, L., K. Elwinger, A. Lundeń, P. Thebo, M. R. Bedford, and A. Uggla. 2000. Intestinal digesta viscosity decreases during coccidial infection in broilers. Br. Poult. Sci. 41:459–464.

Yun, C.-H., A. Estrada, A. van Kessel, A. A. Gajadhar, M. J. Redmond, and B. Laarveld. 1997. β-(1→3, 1→4) Oat glucan enhances resistance to *Eimeria vermiformis* infection in immunosuppressed mice. Int. J. Parasitol. 27:329–337.

Yun, C.-H., A. Estrada, A. Van Kessel, B.-C. Park, and B. Laarveld. 2003. β-Glucan, extracted from oat, enhances disease resistance against bacterial and parasitic infections. FEMS Immunol. Med. Microbiol. 35:67–75.