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Original Research Article

Diet medication and beta-glucanase affect ileal digesta soluble beta-glucan molecular weight, carbohydrate fermentation, and performance of coccidiosis vaccinated broiler chickens given wheat-based diets

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ABSTRACT

Exogenous enzymes as alternatives to feed antibiotics in poultry has become an emerging research area with the emergence of antibiotic resistance. The objective was to evaluate the effects of diet medication (antibiotics) and β-glucanase (BGase) on digesta soluble β-glucan depolymerization, carbohydrate fermentation, and performance of coccidiosis-vaccinated broiler chickens fed wheat-based diets. A total of 1,782 broilers were raised on litter floor pens, and each treatment was assigned to 1 pen in each of the 9 rooms. The 3 dietary treatments were based on wheat as the sole grain (control, control + medication and control + 0.1% BGase), and the birds were fed the respective treatments ad libitum from 0 to 33 d. Treatments were arranged in a randomized complete block design and analyzed as a one-way ANOVA. Beta-glucanase reduced the peak molecular weight, weight average molecular weight (Mw) and maximum molecular weight for the smallest 10% β-glucan molecules (MW-10%) in ileal digesta at d 11 and 33, whereas diet medication reduced Mw and MW-10% at d 33 compared to the control ($P < 0.01$). Beta-glucanase and medication reduced the ileal viscosity at d 11 compared to the control ($P = 0.010$). Ileal propionic acid concentration at d 11 and caecal total SCFA, acetic, and butyric acid concentrations at d 33 were lower in the BGase-supplemented diet than in the control ($P < 0.05$). The BGase-added diet had higher duodenal pH compared to the control at d 33 ($P = 0.026$). The effect of medication on carbohydrate fermentation was minimal. Diet medication increased weight gain after d 11, whereas BGase increased the gain for the total trial period compared to the control ($P < 0.001$). Feed intake was not affected by the dietary treatment. Medication and BGase improved feed efficiency after d 11 compared to the control ($P < 0.001$). The response to diet medication was larger than BGase, considering weight gain and feed efficiency after d 11 ($P < 0.001$). In conclusion, diet medication and BGase depolymerized high molecular weight ileal soluble β-glucan and increased overall bird performance. Dietary BGase may benefit bird health in broilers fed wheat-based diets without medication.

1. Introduction

Poultry feed antibiotics have been reduced or banned due to the emergence of antibiotic-resistant bacterial genes in poultry (Bean-Hodgins and Kiarie, 2021), which affects both animals and public health, and is also a significant food safety issue due to the potential drug residues in poultry-based food products (Ma et al., 2021). However, the reduction of antibiotics in poultry feed leads to the

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increased susceptibility of chickens to enteric and zoonotic diseases, which in turn has led to the investigation of alternative approaches to preventive antibiotic use (Abd El-Hack et al., 2022; Zhu et al., 2021).

Wheat is commonly used as a poultry feed ingredient due to its relatively high energy and crude protein content (Pirgozliev et al., 2015). The nutritional value of wheat is enhanced by supplementing dietary endo-xylanase, which depolymerizes wheat arabinoxylan into arabinoxylo-oligosaccharides and xylo-oligosaccharides (Bautil et al., 2019). The depolymerization of wheat arabinoxylan increases digestive tract short chain fatty acid (SCFA) production and bird performance and minimizes susceptibility to enteric diseases in poultry given diets without antibiotics and anti-coccidial drugs (Bautil et al., 2021; Courtin et al., 2008; Kiarie et al., 2014; Morgan et al., 2019). The rationale for medication-induced growth performance and reduced predisposition to enteric disease is not recognized. Although an increase in digestive tract SCFA has been speculated to cause these beneficial effects, Ribeiro et al. (2018) concluded that a limited increase in SCFA did not affect broiler performance. This is supported by the finding that feeding a small quantity of xylo-oligosaccharides with minimal effects on digestive tract fermentation positively affected bird performance (Ribeiro et al., 2018). It has been hypothesized that the xylo-oligosaccharides signal the gut microbiota to utilize dietary fiber more effectively by increasing microbial xylanase and cellulase activities and increasing overall gut health and production performance (Dale et al., 2020; Ribeiro et al., 2018).

Beta-glucan is a dietary fiber from wheat other than arabinoxylan, although it is found at much lower levels (β-glucan, 0.19% to 0.67%; arabinoxylan, 4.95% to 6.25%) (Havrlentova and Kraic, 2006; Karunaratne et al., 2018). Cereal β-glucan hydrolysis has been shown to enhance immune response (Estrada et al., 1997; Yun et al., 2003) and have beneficial effects on the performance of coccidiosis vaccinated broiler chickens fed antibiotic-free hulless barley-based diets (Karunaratne et al., 2021b, 2021c). However, it is interesting to understand the effects of the low levels of β-glucan and their hydrolysis on the performance of broiler chickens fed wheat-based diets with purified β-glucanase (BGase). Most of the wheat and exogenous enzyme research has used either endo-xylanase and BGase activity or only endo-xylanase activity (Clarke et al., 2018; Mathlouthi et al., 2002; Munyaka et al., 2016). This paper presents the first research to use a purified BGase in wheat-fed broiler chickens. Evaluating the single effect of BGase, rather than an enzyme having both BGase and xylanase activities, offers the opportunity to study changes in β-glucan molecular weight and microbial fermentation in the digestive tract, as well as how enzyme addition affects broiler performance, since wheat has a low level of β-glucan, but mostly arabinoxylan.

How feed medication improves bird performance is not well understood, although antibiotics in feed have been effectively used to enhance gastrointestinal health and production performance in broiler chickens (Barton, 2000; Mehdi et al., 2018). It is generally acknowledged that antibiotics in feed positively influence gut microbial diversity and relative abundance (Costa et al., 2017; Singh et al., 2013) and thereby minimize the predisposition to enteric diseases. The examination of feed medication and enzyme effects in fiber-containing diets provides an opportunity to enhance our understanding of the mechanism of action of feed antibiotics on the molecular weight of digesta β-glucan possibly through the alteration of gut microbiota, and study the possibility of partially replacing antibiotics with exogenous fiber-degrading enzymes in broiler diets. The prebiotic effect of wheat β-glucan can be evaluated by studying the effect of BGase and medication on digesta β-glucan hydrolysis (β-glucan molecular weight analysis) and the impact of the resulting β-glucan oligosaccharides on microbial fermentation and bird performance. The study excludes a medication and enzyme combined treatment diet as the research objective focuses on comparing feed medication and enzymes in a wheat-based broiler diet to evaluate the possibility of BGase as an alternative to antibiotics. It is imperative to study the effects of feed antibiotics and BGase on bird performance and carbohydrate fermentation through digesta β-glucan hydrolysis under a disease-challenged condition to understand the mechanism of action of both diet medication and the enzyme. Coccidiosis is a commonly encountered enteric disease in broiler chickens; therefore, the vaccination of all the birds against coccidiosis in the current study would facilitate the objective of the research.

The objective of the current research was to evaluate the effects of a wheat-based diet with and without medication or BGase on digesta soluble β-glucan molecular weight, ileal and caecal carbohydrate fermentation, and performance of broiler chickens vaccinated for coccidiosis. It was hypothesized that BGase would depolymerize wheat β-glucan, increase digestive tract carbohydrate fermentation (increasing SCFA concentrations and reducing digesta pH in the hind gut), and improve broiler performance. If correct, this would suggest BGase use in broiler wheat diets as an alternative (or partial alternative) to in-feed antibiotics.

2. Materials and methods

2.1. Animal ethics

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

2.2. Birds and housing

A total of 1,782 broiler chickens (Ross × Ross 308) at 1 d of age obtained from a commercial hatchery were randomly placed in 27 floor pens (2.3 m length × 2 m width) in 9 environmentally controlled rooms. In each floor pen, 33 males and 33 females were placed at an estimated final density of 31 kg/m^2. Each of the 3 dietary treatments was randomly assigned to 1 pen per room. Straw was placed on the floor of each broiler pen, and the thickness was 7.5 to 10 cm. Room temperature was 33 °C on d 0 and then gradually decreased until it was 21 °C by d 25. Light intensity was set at 20 lux at the time of chick placement and progressively reduced to 10 lux by d 10. Day length was 23 h at the beginning of the trial, and it was gradually reduced to 17 h by d 12. Feed was provided ad libitum using a tube feeder having a pan diameter of 36 (0 to 25 d) or 43 cm (>25 d) in each pen. Each pen was equipped with a height-adjustable nipple drinker, and each drinker contained 6 Lubing nipples. A cardboard egg tray and an ice cube tray were placed on each pen as supplementary feeders and drinkers for the first 7 d until they adapted to the primary feeders and drinkers.

2.3. Experimental diets

The control diet was 60% wheat-based. The other 2 treatment diets were made by adding either BGase (Econase GT 200 P from AB Vista, Wiltshire, UK) at 0.1% (BGase activity; 200,000 BU/kg) or medication (4.4 mg/kg bacitracin [Zoetis Canada Inc., Kirkland, QC, Canada] and 25 mg/kg salinomycin sodium [Phibro Animal Health Corporation, Teaneck, NJ]) to the control diet. Beta-glucanase level in the diet was selected as 0.1% due to the requirement of a

relatively high enzyme level to increase the probability of β-glucan depolymerization and consequential positive effects. The rations were prepared according to Ross 308 broiler nutrition specifications (Aviagen, 2014). The feed ingredient composition and estimated nutrient levels are presented in Table 1. Starter diets were fed from 0 to 11 d, and grower diets were given from 11 to 33 d. The starter diets were in crumble form, and the grower diets were first in crumble form and then shifted to pellet form. The pellet conditioner temperature was maintained between 70 and 75 °C to protect BGase from high-temperature-induced destruction. Diet BGase (EC 3.2.1.6) and xylanase (EC 3.2.1.8) activities were evaluated using AB Vista methods of ESC Standard Analytical Method SAM042-01 and SAM038, respectively. Xylanase activity in the diets was non-measurable (<2,000 U/kg), and BGase activity approached the anticipated enzyme activity (control, 19,100 BU/kg; control + diet medication, 17,900 BU/kg; control + BGase, 89,300 BU/kg). Beta-glucanase activity of Econase GT 200 P was 273,000 BU/kg, and xylanase activity was non-detectable.

All the birds were challenged with the Coccivac B-52 live vaccine (Merck Animal Health, Madison, NJ), consisting of *Eimeria acervulina, E. mivati, E. tenella* and 2 strains of *E. maxima* oocysts. Vaccination of 1.3 times the recommended dose was completed at 5 d of age to enable uniform oocyst intake, according to Karunaratne et al. (2021b). The birds were not clinically infected as the total mortality of the trial was 4.2%, and the clinical signs related to coccidiosis were absent. However, subclinical coccidiosis was confirmed due to the observation of *Eimeria*-associated intestinal mucosal lesions during sample collection.

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

Item	Starter (d 0 to 11)	Grower (d 11 to 33)
Ingredient		
Wheat[1]	59.09	64.76
Soybean meal	32.97	26.93
Canola oil	3.29	4.07
Mono-dicalcium 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
Calculated nutrient		
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
Non-phytate phosphorous	0.48	0.44
Potassium	0.92	0.83
Sodium	0.20	0.18
Digestible arginine	1.50	1.34
Digestible isoleucine	0.90	0.81
Digestible leucine	1.61	1.46
Digestible lysine	1.28	1.15
Digestible methionine	0.59	0.54
Digestible methionine + cystine	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, 14.4; insoluble dietary fiber, 12.4; soluble dietary fiber, 2.0; β-glucan, 0.64 (%, DM basis).

[2] Vitamin-mineral premix offered 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.

2.4. Performance data and sample collection

Feed intake and body weight were taken on a pen basis at d 11, 22, and 32, and body weight gain (BWG) and mortality adjusted feed to gain ratio (F:G) were determined. Mortality was recorded daily, and bird cadavers were forwarded to Prairie Diagnostic Services at the University of Saskatchewan for post-mortem examination.

Two birds from each pen were randomly sampled, and individual weights were taken at d 11 and 33. The birds with extreme body weights (±15% average body weight) were substituted during weighing. The selected birds were sacrificed by injecting T-61 euthanasia solution (Merck Animal Health, Kirkland, Quebec, Canada) into the brachial vein. Samples for SCFA analysis (6 replicates at d 11; 9 replicates at d 33) were collected, and pH measurements (9 rooms) were taken from both birds. In situ pH was assessed in the contents of the crop, gizzard, duodenum, jejunum, ileum, caeca and colon using a Beckman Coulter 34 pH meter (Model PHI 34, Beckman Instruments, Fullerton, CA). Total ileal and caecal contents for SCFA analysis were collected into plastic centrifuge tubes and stored at −20 °C. A fraction of the pooled ileal content was compiled 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). The viscosity of the pooled ileal supernatant (6 rooms at d 11; 9 rooms at d 33) was evaluated utilizing a Brookfield cone-plate viscometer (Model LVDV-III, Brookfield Engineering Labs, Inc., Stoughton, MA 02072), retained at 40 °C (shear rate 300 s^{-1}). The remaining ileal supernatant was stored at −80 °C for β-glucan molecular weight assessment.

2.5. Dietary nutrient analysis

The diets were ground using a Retsch laboratory mill (Retsch ZM 200, Germany) to 1- and 0.5-mm screen-hole sizes. Analyses, including moisture, total starch, crude protein, fat, ash, total β-glucan, and insoluble and soluble fiber of the ground feed, was completed utilizing the methods and equipment described by Karunaratne et al. (2021c).

2.6. Beta-glucan molecular weight of ileal soluble digesta

Ileal supernatant was evaluated for β-glucan molecular weight using size exclusion chromatography with calcofluor post-column detection for fluorescent recognition (Karunaratne et al., 2021c). Peak molecular weight (Mp) identifies the molecular weight of the highest β-glucan fraction. Weight average molecular weight (Mw) characterizes the average of all the molecular weights of β-glucan (based on the weight fraction of the molecules). The maximum molecular weight for the smallest 10% β-glucan molecules (MW-10%) was also reviewed. The molecular weight demarcation of the smallest 10% β-glucan molecules in the molecular weight distribution curve (number of molecules against molecular weight) is called MW-10%.

2.7. Short chain fatty acids analysis

Short chain fatty acids were analyzed in ileum and caeca using the method described by Karunaratne et al. (2021a). The analysis was completed using a Thermos Scientific gas chromatography system (Model Trace 1310, Milan, Italy) equipped with a Zebron capillary gas chromatography column (Zebron ZB-FFAP, Phenomenex, Torrance, CA) and a flame ionization detector.

2.8. Statistical analysis

The experiment was designed according to a randomized complete block design with a room used as a block to minimize

possible environmental differences among rooms. Data were tested for normality by the Shapiro–Wilk test and analyzed using a one-way ANOVA of JMP software from the SAS Proc GLM model (JMP Inc., Cary, NC). The significance level was defined at $P \leq 0.05$, and the trends were considered when $0.05 < P \leq 0.10$. Mean separation was completed using the Tukey–Kramer test.

3. Results

3.1. Nutrient composition

The total dietary fiber, insoluble dietary fiber, soluble dietary fiber and total β-glucan in wheat were 14.4%, 12.4%, 2.0% and 0.64% (DM basis), respectively (Table 1). The total starch, CP, fat, and ash contents of wheat were analyzed as 62.8%, 14.9%, 1.2%, and 1.7%, respectively. The analyzed nutrient compositions of the experimental diets are shown in Table 2.

3.2. Beta-glucan molecular weight of ileal soluble digesta

Broiler chickens fed diets without BGase resulted in higher ileal digesta soluble β-glucan Mp ($P = 0.003$), Mw ($P < 0.001$) and MW-10% ($P < 0.001$) values at d 11 than those fed the diet with BGase (Table 3). Diet medication did not reduce the molecular weight parameters in ileal digesta compared to the wheat-based control diet at d 11. At d 33, the broilers given medicated or BGase-added diets showed lower Mw ($P = 0.001$) and MW-10% ($P < 0.001$) values in ileal digesta than birds fed wheat-based control diets. However, Mp was reduced only by dietary BGase compared to the control diet ($P = 0.003$).

3.3. Ileal viscosity

At d 11, ileal viscosity was higher in the broilers fed the control diet than in the diets containing medication or BGase ($P = 0.010$; Table 3). There were no significant differences among treatments for the ileal viscosity of broiler chickens at d 33 ($P = 0.232$).

3.4. Short chain fatty acids and gastrointestinal pH

Ileal propionic, valeric, isovaleric and caproic acid concentrations were affected by dietary treatment at d 11 (Table 4). The ileal propionic acid concentration was lower in the BGase-added diet than in the control diet; the medicated diet was not significantly different from the other 2 treatments ($P = 0.037$). Isovaleric ($P = 0.028$), valeric ($P = 0.002$) and caproic acid ($P = 0.015$) concentrations in the ileum were lower in the birds fed the medicated diet than those fed the control or BGase diets. Acetic ($P = 0.001$) and lactic acid ($P = 0.014$) molar percentages in the ileum were higher in the medicated treatment than in the control and BGase treatments. The molar percentages of ileal valeric ($P < 0.001$) and caproic acids ($P = 0.005$) were lower in the birds fed medicated diet than in the other 2 diets. The BGase-supplemented diet resulted in a higher isovaleric acid percentage than the medicated diet in the ileum ($P = 0.030$). Short chain fatty acids (concentrations and percentages) in the caeca of 11-d-

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

Item	Wheat	Wheat + medication	Wheat + β-glucanase
Starter diets			
Moisture	6.9	6.6	6.6
Total starch	38.5	37.0	35.1
Crude protein	25.5	25.8	26.7
Ether extract	3.7	4.3	4.0
Ash	5.6	6.4	6.1
Total dietary fiber	23.4	24.2	24.8
Insoluble dietary fiber	19.3	19.4	19.4
Soluble dietary fiber	4.1	4.8	5.3
Total β-glucan	1.0	0.8	0.8
Grower diets			
Moisture	6.3	6.3	6.1
Total starch	40.1	38.8	38.7
Crude protein	23.1	23.4	22.9
Ether extract	4.9	4.9	4.6
Ash	5.6	5.6	5.6
Total dietary fiber	23.3	24.5	26.8
Insoluble dietary fiber	20.3	20.6	22.4
Soluble dietary fiber	3.0	3.8	4.5
Total β-glucan	0.6	0.6	0.9

Table 3
Beta-glucan molecular weight and viscosity in the soluble ileal content of broiler chickens (g/mol).

Diet	Day 11				Day 33			
	Mp	Mw	MW-10%	Viscosity, cP	Mp	Mw	MW-10%	Viscosity, cP
Wheat	36,397[a]	41,886[a]	19,645[a]	7.84[a]	36,663[a]	42,414[a]	20,256[a]	2.37
Wheat + medication	37,480[a]	40,117[a]	20,909[a]	3.17[b]	31,059[ab]	35,681[b]	12,727[b]	2.69
Wheat + β-glucanase	31,029[b]	29,771[b]	10,449[b]	3.36[b]	25,983[b]	30,527[b]	9,757[b]	2.83
SEM[1]	807.9	1,186.9	690.1	1.051	1,638.7	1,650.3	1,088.4	0.187
ANOVA *P*-value	0.003	<0.001	<0.001	0.010	0.003	0.001	<0.001	0.232

Mp = peak molecular weight; Mw = weight average molecular weight; MW-10% = the maximum molecular weight for the smallest 10% β-glucan molecules.
[a–b]Means within a column not sharing a common superscript are significantly different ($P \leq 0.05$).
[1] SEM = pooled standard error of the mean (molecular weight, $n = 6$ birds per treatment; viscosity (d 11), means of 6 replications; viscosity (d 33), means of 9 replications. Pooled ileal samples from 2 birds per replicate in each day).

Table 4
Ileal short chain fatty acids of broiler chickens at 11 d of age.

Diet	SCFA, μmol/g of wet ileal content								Molar percentage of total SCFA, mol/100 mol						
	Total	Ace	Pro	Buty	Val	Isov	Cap	Lac	Ace	Pro	Buty	Val	Isov	Cap	Lac
Wheat	126.1	48.0	18.5^{a}	8.2	2.4^{a}	2.7^{a}	1.1^{a}	44.9	38.0^{b}	14.7	6.5	1.9^{a}	2.1^{ab}	0.9^{a}	35.6^{b}
Wheat + medication	121.9	47.8	18.1^{ab}	8.1	0.9^{b}	1.8^{b}	0.7^{b}	44.2	39.2^{a}	14.8	6.6	0.7^{b}	1.5^{b}	0.6^{b}	36.2^{a}
Wheat + β-glucanase	118.0	44.9	16.8^{b}	7.8	2.5^{a}	2.6^{ab}	1.1^{a}	42.0	38.0^{b}	14.3	6.5	2.1^{a}	2.2^{a}	0.9^{a}	35.6^{b}
SEM[1]	3.81	1.56	0.45	0.30	0.27	0.21	0.11	1.39	0.19	0.16	0.05	0.21	0.17	0.08	0.16
ANOVA *P*-value	0.323	0.300	0.037	0.618	0.002	0.028	0.015	0.316	0.001	0.106	0.113	<0.001	0.030	0.005	0.014

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.
[a–b]Means within a column not sharing a common superscript are significantly different ($P \leq 0.05$).
[1] SEM = pooled standard error of the mean ($n = 12$ birds per treatment).

old broiler chickens were not affected by the treatment diet except valeric acid at 11 d (Table 5). The medicated diet resulted in higher caecal valeric acid concentration than the control diet ($P = 0.023$). The caecal valeric acid percentage was higher in medicated and BGase-added diets than in the control diet ($P = 0.021$). Short chain fatty acid concentrations and molar percentages in the ileum were not affected by the treatment diet at d 33 (Table 6). At d 33, total SCFA ($P = 0.048$), acetic ($P = 0.044$) and butyric acid ($P = 0.036$) concentrations in the caeca were lower in the BGase-supplemented diet than in the control diet (Table 7). The treatment diet did not affect molar percentages of SCFA in the caeca at d 33. The BGase diet had the highest duodenum pH, whereas the lowest was observed for the wheat-based control diet; the medicated diet had an intermediate value at d 33 ($P = 0.026$) (Table 8).

3.5. Performance parameters

Treatment diets affected BWG and the F:G at all the evaluated periods (Table 9). The BGase treatment increased BWG compared to the medicated diet in broiler chickens from 0 to 11 d, whereas the control diet resulted in an intermediate value not statistically different than the other treatments ($P = 0.018$). The medicated diet resulted in higher BWG than the BGase and control diets during 11 to 22 d ($P < 0.001$) and 22 to 32 d ($P < 0.001$). Over the total study period (0 to 32 d), the BWG ranking from highest to lowest was wheat + medication, wheat + BGase, and wheat without supplements, and all the differences were statistically significant ($P < 0.001$). Feed intake was not affected by treatment diets at all the study periods.

From 0 to 11 d, the BGase diet decreased the broiler F:G compared to the medicated diet, whereas the value for the control diet was intermediate and not different from the others ($P = 0.002$). From d 11 onwards and for the total trial period, the medicated diet resulted in the lowest F:G, and the highest F:G was found for the control diet ($P < 0.001$). The BGase-supplemented diet showed intermediate values regarding F:G for the same periods. For all of the latter F:G comparisons, all differences were statistically significant.

The average mortality for the trial was 4.2%, and death loss was not affected by dietary treatment. The birds did not have clinical coccidiosis as the total mortality was low and there were no clinical signs. However, gross pathological lesions of *Eimeria* spp. were observed in the euthanized birds during the sample collection, indicating subclinical coccidiosis of the flock due to the coccidiosis vaccination.

Table 5
Caecal short chain fatty acids of broiler chickens at 11 d of age.

Diet	SCFA, μmol/g of wet caecal content								Molar percentage of total SCFA, mol/100 mol						
	Total	Ace	Pro	Buty	Isob	Val	Isov	Cap	Ace	Pro	Buty	Isob	Val	Isov	Cap
Wheat	266.0	157.1	58.0	26.4	8.6	3.4^{b}	8.6	3.7	58.9	21.7	9.9	3.2	1.3^{b}	3.2	1.3
Wheat + medication	237.6	137.4	50.7	23.6	7.5	7.5^{a}	7.5	3.2	57.8	21.3	9.9	3.1	3.1^{a}	3.1	1.3
Wheat + β-glucanase	217.9	126.8	46.2	21.3	6.9	6.8^{ab}	6.8	2.9	58.0	21.2	9.8	3.1	3.1^{a}	3.1	1.3
SEM[1]	31.99	19.46	6.91	3.10	1.02	1.38	1.01	0.43	0.47	0.30	0.09	0.03	0.52	0.04	0.01
ANOVA *P*-value	0.263	0.298	0.223	0.258	0.222	0.023	0.224	0.225	0.257	0.271	0.076	0.107	0.021	0.292	0.237

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.
[a–b]Means within a column not sharing a common superscript are significantly different ($P \leq 0.05$).
[1] SEM = pooled standard error of the mean ($n = 12$ birds per treatment).

Table 6
Ileal short chain fatty acids of broiler chickens at 33 d of age.

Diet	SCFA, μmol/g of wet ileal content									Molar percentage of total SCFA, mol/100 mol							
	Total	Ace	Pro	Buty	Isob	Val	Isov	Cap	Lac	Ace	Pro	Buty	Isob	Val	Isov	Cap	Lac
Wheat	121.3	46.6	17.5	7.9	0.1	2.6	2.4	1.1	42.8	38.4	14.5	6.5	0.1	2.1	2.0	0.9	35.3
Wheat + medication	123.8	47.3	17.9	8.0	0.0	2.6	2.6	1.1	43.8	38.2	14.5	6.5	0.0	2.1	2.1	0.9	35.4
Wheat + β-glucanase	124.5	47.7	18.1	8.1	0.0	2.6	2.5	1.1	44.2	38.3	14.5	6.5	0.0	2.1	2.0	0.9	35.4
SEM[1]	3.00	1.13	0.43	0.20	0.08	0.06	0.14	0.02	1.07	0.06	0.02	0.01	0.06	0.01	0.10	0.01	0.07
ANOVA *P*-value	0.733	0.777	0.678	0.809	0.353	0.665	0.541	0.684	0.662	0.186	0.458	0.365	0.353	0.339	0.564	0.434	0.342

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.
[1] SEM = pooled standard error of the mean ($n = 18$ birds per treatment).

Table 7
Caecal short chain fatty acids of broiler chickens at 33 d of age.

Diet	SCFA, μmol/g of wet caecal content								Molar percentage of total SCFA, mol/100 mol						
	Total	Ace	Pro	Buty	Isob	Val	Isov	Cap	Ace	Pro	Buty	Isob	Val	Isov	Cap
Wheat	229.4[a]	132.5[a]	49.1	22.9[a]	7.3	7.1	7.2	3.0	57.6	21.4	10.0	3.1	3.1	3.1	1.3
Wheat + medication	197.4[ab]	115.5[ab]	41.3	19.8[ab]	5.7	6.1	6.1	2.6	58.5	20.9	10.0	2.9	3.0	3.1	1.3
Wheat + β-glucanase	197.3[b]	114.8[b]	41.6	19.6[b]	6.2	6.1	6.1	2.6	58.1	21.1	9.9	3.1	3.1	3.1	1.3
SEM[1]	13.88	8.17	2.94	1.35	0.55	0.43	0.43	0.18	0.40	0.24	0.11	0.15	0.03	0.03	0.01
ANOVA *P*-value	0.048	0.044	0.059	0.036	0.222	0.071	0.070	0.074	0.195	0.155	0.981	0.376	0.134	0.124	0.177

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.
[a–b]Means within a column not sharing a common superscript are significantly different ($P \leq 0.05$).
[1] SEM = pooled standard error of the mean ($n = 18$ birds per treatment).

Table 8
Gastrointestinal pH of broiler chickens.

Diet	Day 11						Day 33					
	Crop	Giz	Duo	Jej	Ileum	Caeca	Crop	Giz	Duo	Jej	Ileum	Caeca
Wheat	4.58	2.67	6.00	5.87	6.26	6.03	4.92	3.35	5.78[b]	5.92	6.50	6.37
Wheat + medication	5.00	2.70	5.92	5.93	6.56	5.99	4.78	3.56	6.02[ab]	6.00	6.70	6.31
Wheat + β-glucanase	4.79	2.69	6.00	5.95	6.23	5.90	5.02	3.37	6.06[a]	6.04	6.66	6.21
SEM[1]	0.123	0.115	0.049	0.038	0.101	0.103	0.104	0.101	0.077	0.040	0.164	0.102
ANOVA *P*-value	0.063	0.981	0.453	0.289	0.060	0.649	0.261	0.270	0.026	0.104	0.665	0.531

Giz = gizzard; Duo = duodenum; Jej = jejunum.
[a–b]Means within a column not sharing a common superscript are significantly different ($P \leq 0.05$).
[1] SEM = pooled standard error of the mean ($n = 18$ birds per treatment).

Table 9
Production performance of broiler chickens.

Diet	Body weight gain, kg				Feed intake, kg				Feed to gain ratio			
	Day 0 to 11	Day 11 to 22	Day 22 to 32	Day 0 to 32	Day 0 to 11	Day 11 to 22	Day 22 to 32	Day 0 to 32	Day 0 to 11	Day 11 to 22	Day 22 to 32	Day 0 to 32
Wheat	0.28[ab]	0.64[b]	0.96[b]	1.88[c]	0.34	1.04	1.63	3.00	1.20[ab]	1.49[a]	1.66[a]	1.52[a]
Wheat + medication	0.27[b]	0.71[a]	1.03[a]	2.01[a]	0.34	1.04	1.59	2.98	1.23[a]	1.35[c]	1.53[c]	1.42[c]
Wheat + β-glucanase	0.29[a]	0.67[b]	0.98[b]	1.93[b]	0.34	1.04	1.58	2.96	1.18[b]	1.43[b]	1.60[b]	1.47[b]
SEM[1]	0.003	0.007	0.008	0.014	0.003	0.009	0.013	0.022	0.009	0.014	0.010	0.007
ANOVA *P*-value	0.018	<0.001	<0.001	<0.001	0.482	0.814	0.073	0.504	0.002	<0.001	<0.001	<0.001

[a–c]Means within a column not sharing a common superscript are significantly different ($P \leq 0.05$).
[1] SEM = pooled standard error of the mean (means of 9 replications).

4. Discussion

The analyzed BGase activities of the control and medicated diet (the two diets without any added BGase) were higher than the expected value, which was zero, and it could be attributed to the endogenous BGase activity of the cereal grains (Cardoso et al., 2014). In previous research with broiler chickens (Karunaratne et al., 2021b), the diet β-glucan peak molecular weight value (762×10^3) in 60% hulless barley diets was higher than the values observed in the ileal digesta (78×10^3) without the addition of dietary BGase. The lower than calculated BGase activity in the 0.1% BGase diet might be associated with the impact of heat treatment during the feed pelleting process.

Previous research assessed graded dietary BGase on soluble ileal β-glucan molecular weight in broiler chickens given non-medicated diets containing different levels (0%, 30% and 60%) of hulless barley by replacing wheat (Karunaratne et al., 2021a, 2021b). Therefore, the two non-medicated diets used in the current study were similar to two diets that consisted of 0% hulless barley (60% wheat) in Karunaratne et al. (2021b), and the data, including β-glucan molecular weight, were comparable. Broiler growth performance and digestive tract physiology data, including SCFA, digestive tract pH, and ileal viscosity, were also similar in the current study and Karunaratne et al. (2021b, 2021c) concerning the two treatment diets mentioned above containing 60% wheat. However, the broad comparison and the repeated use of these two treatments and respective data in Karunaratne et al. (2021b, 2021c) are essential in the current study for the distinctive evaluation of dietary BGase and medication effects of wheat-containing diets on β-glucan molecular weight, growth performance and carbohydrate fermentation in broiler chickens. Karunaratne et al. (2021a) also used 60% wheat-containing diets similar to the two non-medicated dietary treatments in the current research; nevertheless, the broilers in the previous study were reared under different management conditions as the birds were not vaccinated for coccidiosis and were raised in battery cages. Therefore, the data of Karunaratne et al. (2021a) were dissimilar to the present study despite the use of comparable diets.

In agreement with our earlier research, feeding BGase reduced the three assessed β-glucan molecular weight parameters in soluble ileal content at both sample collection days for broilers fed a wheat-based diet (Karunaratne et al., 2021a, 2021b). These data confirm that exogenous BGase mediated β-glucan depolymerization in wheat and hulless barley is similar (Karunaratne et al., 2021a, 2021b), despite the differences in β-glucan structure (the ratio of cellotriosyl to cellotetraosyl of β-glucan in wheat and hulless barley is 3.0 to 4.5 and 2.3 to 3.4, respectively, that results in different proportions of tri-saccharides and tetra-saccharides) in

these grains (Biliaderis and Izydorczyk, 2007). Further, the reduction of MW-10% indicates a greater reduction of the molecular weights of the smallest 10% β-glucan proportion in the ileal digesta of birds fed the BGase-supplemented diet compared to the birds given the control diet. It can be anticipated that a higher proportion of relatively smaller molecular weight β-glucan would increase carbohydrate fermentation and stimulate the gastrointestinal microbiota because the gut microbiota in chickens prefer low vs. high molecular weight carbohydrates (Courtin et al., 2008; Lei et al., 2012).

Beta-glucanase depolymerization of β-glucan has also been shown to reduce the viscosity of ileal digesta of broiler chickens (Józefiak et al., 2005, 2006; Salih et al., 1991), and this was also noted in the current research at d 11 but not 33 d of age. The coccidiosis challenge might have altered gut microbiota in broiler chickens at d 11 and reduced ileal viscosity due to the microbial utilization of dietary fiber, including β-glucan (Lu et al., 2021). Viscosity values were lower for all treatments at 33 d of age than 11 d, and this may be attributed to the age-related adaptation of gastrointestinal microbiota to utilize fiber in wheat effectively (Bautil et al., 2019). However, the ileal β-glucan molecular weight values for the control treatment were broadly consistent between the 2 ages. Therefore, the changes in ileal viscosity might be attributed to arabinoxylan rather than β-glucan (Choct and Annison, 1992; Kiarie et al., 2014), the latter being a relatively minor fiber component in wheat. It has been found that a minimal inclusion level of xylanase (lower than the commercial use in poultry feed) reduced the digesta viscosity in broiler chickens significantly (Scott et al., 1998). Despite the undetectable level of xylanase activity in the diets, the endogenous xylanase and the diet-stimulated production of the gut microbial xylanase may have reduced ileal viscosity in the current research (Bautil et al., 2019). Further, BGase might reduce arabinoxylan's molecular weight and viscosity by disrupting the fiber structure. Arabinoxylan and β-glucan are intimately intertwined in the wheat cell wall, and arabinoxylan molecules get detached with the depolymerization of β-glucan associated with arabinoxylan (Philippe et al., 2006). It is also possible that factors other than soluble fiber were responsible for the higher ileal viscosity at 11 d in the control treatment. One such instance is a mild disease that might result from coccidiosis vaccination and increased mucus secretion that enhances digesta viscosity in young birds (Duangnumsawang et al., 2021).

Weight average molecular weight and MW-10% were reduced in broilers given the medicated diet compared to the control diet, but only at 33 d. This response was unanticipated because the medication did not contain BGase activity. However, diet medication might have changed the gastrointestinal microbial population by reducing the pathogenic gut microbiota while increasing the oligosaccharide utilizing beneficial microbes in the digestive tract (Robinson et al., 2019; Xiong et al., 2018). This gut microbial composition and activity shift might affect digesta β-glucan molecular weight by degrading β-glucan into oligosaccharides and low molecular weight polysaccharides (Beckmann et al., 2006). Diet medication-based reduction of soluble ileal digesta β-glucan molecular weight was less (including d 11) than the decline achieved by adding BGase to the diet. The age-dependent development of the gut microbiota in poultry is altered by feed medication since it retards and delays the maturation of the gut microbiome (Gao et al., 2017), which might affect the utilization of dietary fiber, including β-glucan. Therefore, the dietary addition of BGase is required to produce small molecular weight carbohydrates more effectively for fermentation or other biological effects. Medication reduced the ileal digesta viscosity of broiler chickens at d 11 compared to the control, despite the absence of a reduction in ileal soluble β-glucan molecular weight. The diet medication might have prevented the growth of gastrointestinal microbiota that produce small amounts of xylanase, resulting in increased release of insoluble arabinoxylan to reveal soluble, viscous arabinoxylan (Bautil et al., 2019, 2020). Therefore, the reduced viscosity at d 11 could have been due to the reduced population of digestive tract microbes that enzymatically hydrolyze the fibrous cereal cell walls and release the trapped viscous arabinoxylan (Van Hoeck et al., 2021). In the current study, the gut microbiota changes to adapt to a high fiber-containing diet might take an extended time, and coccidiosis vaccination may have also disturbed the gut microbiota composition (Das et al., 2021; Orso et al., 2021). *Eimeria* spp. disrupts the intestinal mucosal structure causing the reduction of commensal bacteria while increasing opportunistic pathogens and thereby reducing the gut microbial potential to evolve its arabinoxylan hydrolysis capacity (Madlala et al., 2021).

The evaluation of carbohydrate fermentation in broiler chickens is crucial as BGase and diet medication reduced the soluble β-glucan molecular weight and increased the proportion of small molecular weight β-glucan in the ileum, which would be a substrate for bacterial fermentation. However, BGase decreased the ileal propionic acid concentration at d 11 and caecal total SCFA, acetic and butyric acids at d 33 compared to the control, indicating the reduction of carbohydrate fermentation in the ileum and caeca, which was unanticipated in the current research. Compared to the control, diet medication decreased ileal valeric, isovaleric, and caproic acid concentrations at d 11 but increased caecal valeric acid concentration at d 11 This may indicate that diet medication has shifted protein fermentation from the ileum to the caeca, which could be related to the shift of the gastrointestinal microbial population, which is a consequence of diet medication on improving gut health in broiler chickens. Microbial fermentation of dietary protein in the small intestine results in ammonia, biogenic amines, phenols, indoles, branched-chain fatty acids, and sulfur-containing compounds (Qaisrani et al., 2015) that detrimentally affect gut health in chickens by increasing pathogenic microbial colonization over beneficial microbes, which eventually negatively affects the production performance of broilers (Gilbert et al., 2018; De Lange et al., 2003).

Body weight gain of the broiler chickens for the total trial period increased with BGase treatment compared to the control, although it was not affected during each particular period. Feed efficiency increased with BGase addition compared to the control during all the periods except from 0 to 11 d. Similar results were observed for the previous studies that used coccidiosis-challenged broiler chickens fed hulless barley-based diets with BGase (Karunaratne et al., 2021b). Dietary BGase (0.1%) did not affect BWG, reduced feed efficiency in the birds aged <11 d, but increased feed efficiency after d 11 (Karunaratne et al., 2021b). Further, diet medication also reduced BWG and feed efficiency in the birds aged <11 d but improved these performance parameters after d 11 in the same study. The reduction of the broiler performance at a young age might be related to the evolution of harmful gastrointestinal microbiota associated with coccidiosis vaccination or the immature digestive system. In the current study, diet medication resulted in higher BWG and feed efficiency than BGase treatment of the birds aged >11 d and for the total trial period. Therefore, BGase did not achieve the same performance by adding medication to broiler diets, although BGase dietary addition increased the performance compared to the wheat-based control diet. Feed medication is a well-established and powerful method of enhancing bird health (Landers et al., 2012). Although many alternatives for medication have been studied, few can replace antibiotics, if any. The use of other non-starch polysaccharide-degrading enzymes, in non-medicated diets, has proven to be successful in production performance by improving the gut health of broiler chickens (Gao et al.,

2008; Saeed et al., 2019). The low molecular weight carbohydrates resulting from exogenous enzyme-induced hydrolysis of dietary polysaccharides have been proven to increase beneficial gut microbiota (Baurhoo et al., 2007; Bogusławska-Tryk et al., 2012; De Maesschalck et al., 2015) and reduce the adverse effects of pathogenic microbiota in the digestive system (Amerah et al., 2012), including the alleviation of intestinal barrier impairment (Liu et al., 2012). However, the research on comparisons between feed antibiotics and enzymes on these effects is minimal, and these fiber-degrading enzymes could not replace feed medication entirely based on the results. The current research suggests that BGase in wheat diets similarly cannot replace antibiotics but may be part of a strategy to maintain bird health without feed medication.

Beta-glucanase and medication in the diets depolymerized high molecular weight β-glucan into oligosaccharides and low molecular weight polysaccharides and increased broiler performance to different extents compared to the broilers given a wheat-based control diet. However, BGase decreased carbohydrate fermentation overall, which is contradictory to the expected outcomes, and the effect of medication on carbohydrate fermentation was minimal based on the SCFA concentrations and digestive tract pH. Therefore, the diet medication and BGase-driven improved performance were not directly related to the quantities of digesta SCFA in broiler chickens. Ribeiro et al. (2018) speculated that a small quantity of low molecular weight carbohydrates might signal gut microbiota to increase the secretion of non-starch polysaccharide degrading enzymes and thereby increase production performance through the utilization of dietary fiber more effectively. Caecal SFCA concentrations were significantly reduced by diet medication (numerically) and BGase at 33 d, and at the same time, these treatments are improving feed efficiency. This might be associated with more undigested nutrients, including starch, in the diet reaching the caeca in the control birds. Further, the current research focused on the concentration of SCFA, but not their rate of production or absorption, which drives the performance response of broiler chickens (Liu et al., 2021).

The current study utilized a purified form of exogenous BGase, and the measured enzyme activity was restricted to BGase in the absence of endo-xylanase, which is different from previous broiler studies (Mathlouthi et al., 2002; Munyaka et al., 2016) that used enzyme products with a combination of enzyme activities (endo-xylanase with BGase and other cellulases) or only endo-xylanase in wheat-based diets. The use of a purified BGase in the current research was essential because the content of β-glucan in wheat is low compared to the amount of arabinoxylan, resulting in a considerable quantity of low molecular weight β-glucan affecting carbohydrate fermentation in broiler chickens fed wheat-based diets.

5. Conclusion

Beta-glucanase and diet medication depolymerized high molecular weight soluble ileal β-glucan into low molecular weight β-glucan, with a lower response observed with the medication compared to BGase. However, treatment only impacted total SCFA in the caeca at d 33, and BGase did not consistently cause reduced SCFA. Diet medication had better FCR than BGase overall and all the time points except the starter period.

Author contributions

Namalika D. Karunaratne: Conceptualization, Methodology, Formal analysis, Investigation, Data Curation, Writing - Original Draft, Visualization. **Henry L. Classen:** Conceptualization, Methodology, Investigation, Resources, Data Curation, Writing - Review & Editing, Supervision, Project administration, Funding acquisition. **Andrew G. van Kessel:** Conceptualization, Methodology. **Michael R. Bedford:** Conceptualization, Methodology. **Nancy P. Ames:** Conceptualization, Methodology, Investigation. **Rex W. Newkirk:** Conceptualization, Methodology, Investigation, Resources, Data Curation, Writing - Review & Editing, Supervision, Project administration, Funding acquisition.

Declaration of competing interest

The authors declare that we have no financial and personal relationships with other people or organizations that can inappropriately influence the current research, with one potential exception, the coauthor and collaborator on this project, Michael R. Bedford, is employed by the company that provided the enzyme used in the research. This coauthor did not bias the research based on his employment status.

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