EFFECTS OF HULLESS BARLEY AND EXOGENOUS BETA-GLUCANASE LEVELS ON BETA-GLUCAN DEPOLYMERIZATION, DIGESTIVE TRACT PHYSIOLOGY AND MORPHOLOGY, AND PERFORMANCE IN CHICKENS

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By

Namalika D. Karunaratne

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ABSTRACT

The reduced use of antibiotics in poultry feed has led to increase enteric disease, and investigation of alternatives to antibiotics has become a major concern. The study evaluated effects of hulless barley (HB) and β-glucanase (BGase) on β-glucan depolymerization, performance, and digestive tract characteristics of chickens. Two broiler trials (a battery cage trial and a floor trial/ coccidiosis challenged birds) and a laying hen trial were conducted by feeding the birds with graded levels of high β-glucan HB and BGase. Exogenous BGase depolymerized high MW β-glucan in the ileum of cage-raised broilers. However, few or no treatment effects noted for digestive tract characteristics and performance. In coccidiosis challenged broilers, BGase depolymerized high MW β-glucan in HB in a dose-dependent manner but had minor effects on digestive tract characteristics. Hulless barley reduced broiler performance and BGase alleviated the effects. However, in young birds fed highest levels of HB and BGase did not affect weight gain while increasing feed efficiency. Overall, HB increased digestive tract size and content, whereas BGase decreased them. In laying hens, BGase depolymerized high MW β-glucan in the ileum in a dose-dependent manner. Effects of medication and BGase were evaluated on broilers fed HB-based diets. Both BGase and medication depolymerized high MW β-glucan in the ileum. Medication and BGase increased the performance in coccidiosis challenged broilers. Effects of HB and BGase were also tested on *Salmonella* colonization in broilers challenged with *Salmonella* Enteritidis. Hulless barley decreased *Salmonella* counts in cloacal swabs and the spleen up to d 1 after the *Salmonella* challenge whereas, increased the counts in spleen after d 1 of the challenge in broiler chickens. However, BGase did not affect *Salmonella* colonization. In conclusion, BGase depolymerized ileal soluble high MW β-glucan in HB in a dose-dependent manner. The resulting low MW β-glucan increased performance in older birds, although no improvement or a reduction of performance was observed in young broilers. The effects of HB and BGase on digestive tract physiological measurements were minor and inconsistent across the experiments. It appears BGase can partially replace antibiotics in HB-based broiler diets.

Keywords: β-glucan, prebiotics, fermentation, feed enzymes, gut health

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DEDICATION

To my mother and father for their enormous love, patience, support, and encouragement to accomplish my dreams

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LIST OF ABBREVIATIONS

µl	Microlitre
µM	Micro moles per litre
AME	Apparent metabolizable energy
AME_n	Nitrogen corrected apparent metabolizable energy
ANR	Apparent nitrogen retained
AvBD	Avian beta-defensin
AX	Arabinoxylan
AXOS	Arabinoxylo-oligosaccharides
BGase	Beta-glucanase
BWG	Body weight gain
Cal	Calories
cDNA	Complementary deoxyribonucleic acids
cm	Centimetres
cP	Centipoise
CP	Crude protein
d	Day
DM	Dry matter
DP	Degree of polymerization
F:G	Feed to gain ratio
FI	Feed intake
FOS	Fructose-oligosaccharides
g	Grams
GAPDH	Glyceraldehyde 3-phosphate dehydrogenase
GC	Gas chromatography
GE	Gross energy

GI	Gastro-intestinal
GIT	Gastro-intestinal tract
GLP-1	Glucagon-like peptide-1
h	Hours
HB	Hulless barley
HDP	Hen day production
HHP	Hen housed production
HPLC	High performance liquid chromatography
IDF	Insoluble dietary fibre
IFN	Interferon
IL	Interleukin
kg	Killograms
l	Litres
LB	Luria-Bertani
LPS	Lipopolysaccharides
m	Metre
m^2	Square metre
MCT1	Monocarboxylate transporter 1
min	Minutes
ml	Millilitres
MLCK	Myosin light-chain kinase
mm	Millimetre
mol	Moles
MOS	Mannan-oligosaccharides
Mp	Peak molecular weight
mRNA	Messenger ribonucleic acids

MUC	Mucin
MW	Molecular weight
Mw	Weight average molecular weight
N	Nitrogen
NSP	Non starch polysaccharides
PAMP	Pathogen associated molecular patterns
PCNA	Proliferative cell nuclear antigen
PCR	Polymerase chain reaction
PRR	Pattern recognition receptors
PYY	Peptide tyrosine tyrosine
qPCR	Quantitative polymerase chain reaction
RNA	Ribonucleic acids
RPL30	Ribosomal protein L30
rpm	Revolutions per minute
RT	Reverse transcription
s	Seconds
SCFA	Short chain fatty acids
SDF	Soluble dietary fibre
SE	*Salmonella* Enteritidis
SI	Small intestine
TDF	Total dietary fibre
TGF	Transforming growth factor
Ti	Titanium
TiO_2	Titanium oxide
TJ	Tight junctions
TLR	Toll-like receptors

TNF	Tumor necrosis factor
U	Units
VFA	Volatile fatty acids
XOS	Xylo-oligosaccharides

1.0 INTRODUCTION

Antibiotics have been added to poultry diets to maintain health and production efficiency of chickens for many decades (Engberg et al., 2000; McEwen and Fedorka-Cray, 2002), but due to the emergence of pathogenic bacterial resistance (Diarra et al., 2007; Furtula et al., 2010; Forgetta et al., 2012; ; Roth et al., 2019) and its impact on public health and food safety (van den Bogaard and Stobberingh, 2000; Marshall and Levy, 2011; Smith et al., 2002), the use of in-feed antibiotics is being reduced. Investigation of alternatives to in-feed antibiotics is a major concern due to the potential for increased enteric disease and reduced performance. As a result, research into alternatives to antibiotics and nutritional impacts on gut health has become a prominent area of research (Diarra and Malouin, 2014; Suresh et al., 2018).

Probiotics, prebiotics, organic acids, essential oils, and feed enzymes are some of the alternative approaches to in-feed antibiotics in the poultry production. The International Scientific Association of Probiotics and Prebiotics defines prebiotic as "a substrate that is selectively utilized by host microorganisms conferring a health benefit" (Gibson et al., 2017). Prebiotics exert health benefits in poultry through a few mechanisms including competitive exclusion of pathogenic bacteria (Ofek and Beachey, 1978; Baurhoo et al., 2007; Kim et al., 2011; Pourabedin et al., 2017), improvement of gastro-intestinal morphology (Xu et al., 2003; Baurhoo et al., 2009; Shang et al., 2015; Ding et al., 2018), production of anti-microbial factors (Chen et al., 2007; Munoz et al., 2012) and modulation of immune function (Babu et al., 2012; Yitbarek et al., 2012; Huang et al., 2015). The most commonly available prebiotics in poultry feed are fermentable carbohydrates. Extensive research has investigated the effects of prebiotics including fructo-oligosaccharides, mannan-olgosaccharides, xylo-oligosaccharides and arabinoxylo-oligosaccharides on poultry health and production performance (Pourabedin and Zhao, 2015; Roberts et al., 2015).

The utilization of dietary constituents with potential prebiotic components has become a significant area of research including the use of non-starch polysaccharide degrading enzymes including endo-xylanase and β-glucanase in the poultry feed industry (Bedford, 2018). Xylanase is commonly added to broiler feed to mitigate the negative effects associated with arabinoxylan (Choct et al., 2004). It is primarily used to reduce the digesta viscosity. However, it has also been

shown to contribute to an increase bacterial fermentation of arabinoxylan, and thereby enhance production performance and gastro-intestinal morphology and physiology in chickens due to the prebiotic properties of the hydrolysis products (Lee et al., 2017; Bautil et al., 2019). The mechanism of endo-xylanase is associated with increased bacterial fermentation through the depolymerization of arabinoxylan into oligosaccharides, which are available as fermentable substrates (Bedford and Classen, 1992). However, less attention has been focused on the addition of feed β-glucanase to high β-glucan cereals, and the subsequent effect of β-glucanase treated feed on carbohydrate fermentation and parameters of gut health in chickens, even though the effects of β-glucanase addition on digesta viscosity is well-established in monogastric animals (Józefiak et al., 2005; 2006).

In the current research, the effects of exogenous β-glucanase and hulless barley on the digestive tract characteristics and performance were evaluated in healthy chickens as well as in diseased birds by using coccidiosis and salmonellosis as disease challenge models. The overall objective of the studies was to evaluate the effects of diet hulless barley and β-glucanase on soluble digesta β-glucan molecular weight distribution, digestive tract physiology and morphology, and production performance of chickens under healthy and disease challenge conditions. It was hypothesized high levels of dietary β-glucanase depolymerize high molecular weight β-glucan in hulless barley into low molecular weight β-glucan, and thereby improve gastro-intestinal physiology and morphology, production performance of chickens by increasing carbohydrate fermentation in the lower digestive tract of chickens.

2.0 LITERATURE REVIEW

2.1. Barley

Barley (*Hordeum vulgare* L.) is classified into three different classes based on its end-use, namely malting barley, food barley, and feed barley. Barley used for brewing is classified as malting barley, and these cultivars are selected based on specific quality standards for that purpose. The Canadian industry has developed a separate and unique class of barley which has been selected for the food market with a focus on high fibre content and/or starch types. These cultivars are mainly used to produce breakfast cereals, rice-like products, thickeners, and food products with elevated levels of β-glucan to create healthier products. There is also a class of barley called feed barley, generally selected for high yields and other agronomic characteristics. In addition, barley that does not meet the quality standards for malting or food is used to feed animals and is graded as feed barley (CGC, 2018). Globally up to 85% of barley produced for malting is used to feed animals including beef, dairy, swine, and poultry (Jacob and Pescatore, 2012).

Barley can also be classified based on the configuration of seeds along the rachis (2-row, 6-row) and presence/absence of a hull. A head of 2-row barley contains two rows of kernels along its length, whereas 6-row barley contains six rows of kernels, in two groups of three kernels each. Covered barley has hull attached to the kernel after harvesting, hulless barley (HB) has a hull that is more loosely attached to the kernel, and therefore detaches during harvesting (CGC, 2018). Other classifications are based on growth season (winter, spring), amylose to amylopectin ratio, aleurone colour (colourless, white and yellow), growth height (tall, semi-dwarf and dwarf) and the presence/absence of an awn. Barley is a short-season, early maturing crop and adapted to a wide variety of climates (Jacob and Pescatore, 2012).

Barley can be included in poultry feed as an energy source but is not well digested due to the presence of non-starch polysaccharides (NSP) and in particularly β-glucan. Barley contains around 60% starch, 22% fibre and 10-12% protein content (Bach Knudsen, 1997; Shewry and Halford, 2002). Barley contains a relatively high level of NSP (4.5% of DM) especially β-1,3-1,4 glucan (3.6% of DM, Cardoso et al., 2014). Aman and Graham (1987) observed a range of 2-10% of total β-1,3-1,4 glucan in barley. The apparent metabolisable energy value of conventional

barley is lower than other cereals due to the high proportion of indigestible fibre that is primarily associated with the hull. However, this insoluble fibre could potentially increase retention in the gizzard and therefore increase overall gut health. Soluble β-glucan which primarily associated with the endosperm rather than the hull increases intestinal viscosity and reduces nutrient utilization in poultry especially if β-glucanase is not added to the diet.

2.1.1. Hulless barley

Plant breeders have developed barley varieties that lose their hull during harvesting and they are termed hulless barley (HB) (McNab and Smithard, 1992). In HB, the hull is less firmly attached to the kernel and then detached during threshing. Because of the loss of a hull, the grain contains a higher level of nutrients and a higher bushel weight/grain density (Classen et al., 1988b). Despite the lower fibre content, the feeding value of HB for poultry remains poor due to the concentration of soluble β-glucan which causes high digesta viscosity (Classen et al., 1985). However, HB is beneficial in terms of controlling human metabolic diseases including type 2 diabetes, cardiovascular diseases, and obesity due to the presence of soluble β-glucan (Wang et al., 2016), and therefore high β-glucan is often a cultivar selection criterion. The total β-glucan and soluble β-glucan contents range from 3.6 to 9.7 and 1.2 to 3.5%, respectively, in HB (Izydorczyk and Dexter, 2008). Of note, there is a health claim about barley products containing high level of β-glucan and blood cholesterol lowering (Health Canada, 2012).

2.2. Cereal β-glucan

2.2.1. Beta-glucan content, structure, and molecular weight

Beta-glucans are defined as linear polymers of D-glucopyranosyl molecules linked by β-type glycosidic bonds. They are originated from the cell walls of a wide range of sources including fungi, yeast, algae, bacteria, and plants. Cereal β-glucans are linear homopolymers of D-glucopyranosyl residues linked mostly via two or three consecutive β-(1-4) linkages that are separated by a single β-(1-3) linkage (Izydorczyk and Dexter, 2008). Long segments of β-(1-4)-linked glucopyranosyl molecules with a degree of polymerization (DP) of 5-28 are rare in cereal β-glucan (Cui et al., 2000; Dais and Perlin, 1982). Fungal, yeast, algae and bacterial cell walls contain β-glucan with a linear β-(1-3)-glucan linked backbone, and it connects to the other β-(1-

3) linked chains via β-(1-6) linkages (Nakashima et al., 2018). Cellulose and mixed linked β-glucan are polymers of β-D-Glucose residues. However, mixed linked β-glucan differs from highly regular β-(1-4) structure of cellulose as β-glucan has both 4-O-linked β-D-glucopyranosyl units (70%) and 3-O-linked β-D-glucopyranosyl units (30%), whereas cellulose contains 4-O-linked β-D-glucopyranosyl units only. The presence of insoluble β-glucan permits close association of part of β-glucan with cellulose or arabinoxylan, whereas soluble β-glucan has no possibility of inter-chain cross-linking, and results in viscous gels (McNab and Smithard, 1992).

There are differences in terms of β-glucan content and structure among cereal grains. Beta-glucan content and structure are influenced by genetic and environmental factors (Bach Knudsen, 2014). The structural differences in β-glucan affect physical properties of the grain including water solubility, dispersibility, viscosity, and physiological effects in the gastrointestinal tract (Biliaderis and Izydorczyk, 2007). Solubility of β-glucan affects digestive tract properties in monogastric animals including digesta viscosity, nutrient digestibility, and microbial fermentation. Total β-glucan content in barley, oats, rye and wheat are 2.5-11.3, 2.2-7.8, 1.2-2 and 0.4-1.4%, respectively (Biliaderis and Izydorczyk, 2007). The β-glucan content in corn and sorghum is around 0.1% (Bach Knudsen, 2014). In barley, 70% of β-glucan is located in the endosperm walls, and around 25% present in the isolated aleurone cell walls (McNab and Smithard, 1992). However, β-glucan is found in the subaleurone layer, other than the aleurone layer and endosperm cell wall in oats (Wang and Ellis, 2014). Beta-glucan content is affected by genetic factors of specific grains, and it is reported barley with normal starch contains higher β-glucan content compared to barley starch with anomalous amylose to amylopectin ratios (Izydorczyk et al., 2000). The structural differences of β-glucan among cereals are associated with β-(1-4)/β-(1-3) linkages, the ratio of cellotriosyl to cellotetraosyl units, molecular size and the presence of long cellulose-like fragments. The differences in the ratio of cellotriosyl to cellotetraosyl units (DP3/DP4) in β-glucan determine the oligosaccharide distribution of cereal β-glucan in the grain and in the digestive tract content when fed to animals. The ranges of β-glucan DP3 to DP4 ratio in barley, oats, rye and wheat are 2.3-3.4, 1.5-2.3, 1.9-3.0 and 3.0-4.5, respectively. The DP3 and DP4 proportions of wheat, barley and oats are reported as 67-72, 52-69 and 53-61, and 21-24, 25-33 and 34-41%, respectively (Biliaderis and Izydorczyk, 2007). It

agrees with the predominant molar proportion which is highest in wheat compared to the other grains analyzed (Cui et al., 2000; Wood et al., 2003). The higher DP3/DP4 ratio in wheat is associated with a more regular structure and poorer β-glucan solubility in comparison to the other grains (Cui et al., 2000). The presence of cellulose-like fragments (DP 5 to 28) in cereal β-glucan can also vary, and in barley, the higher level of the fragments is found in HB cultivars with high amylose to amylopectin ratios (Storsley et al., 2003).

Molecular weight (MW) of β-glucan is also an important factor that is affected by genetic and environmental factors. The average MW of β-glucan in barley, oats, rye and wheat are 31-2700×10^3, 35-3100×10^3, 21-1100×10^3 and 209-416×10^3, respectively (Biliaderis and Izydorczyk, 2007). There are differences in MW within a grain due to the variation of cultivars and growing conditions. The average molecular weight (Mw) of extracted β-glucan of HB has ranged from 800×10^3 to 5900×10^3 Da (Storsley et al., 2003). Peak molecular weight (Mp) of purified β-glucan varies from 800×10^3 to 1500×10^3 Da in barley and oats. In addition, Mp of purified β-glucan from wheat varies from 373×10^3 to 800×10^3 Da (Cui et al., 2000).

The genotype and the growing environment of barley affects β-glucan MW and viscosity. Barley cultivars with low or high proportions of amylose in the starch have been reported to have higher MW β-glucan compared to barley from normal amylose genotypes (Storsley et al., 2003). Beta-glucan may be also become depolymerised prior to consumption by endogenous enzyme activity in grains and other factors such as exposure to acid/alkali and extrusion (Johansson et al., 2007). Furthermore, a high MW distribution of extractable β-glucan has been shown to be maintained by heat and moisture treatment of the grain, which may inactivate endogenous enzymes, and increase the pool of soluble β-glucan with high MW, and consequently barley viscosity (Izydorczyk et al., 2000; Ames et al., 2011; Zhang et al., 2011). In addition, β-glucan MW is also influenced by the extraction technique because of variation in technique temperature, pH, solutions, time and purity of enzymes (Wood et al., 1991; Beer et al., 1997; Knuckles et al., 1997; Zhang et al., 1998; Storsley et al., 2003). The differences in the β-glucan structure and content of cereals might affect the functional properties and physiological effects of β-glucan in monogastric animals including poultry.

2.2.2. Beta-glucan digestibility

There is no data available regarding the digestibility of β-glucan in chickens. However, many studies have measured β-glucan digestibility in pigs using different cereals including barley, oats, and wheat in the feed. Ileal β-glucan digestibility varied from 64 to 88%, while total tract approached 100% when pigs were fed diets containing wheat flour (67.5-79.4%) and wheat fractions (aleurone, pericarp and bran). Ileal and total tract digestibility of β-glucan was found to be 64-73 and 100%, respectively, in pigs fed an oat fractions-based (79.4-89.2%) diet (Bach Knudsen and Hansen, 1991). Similarly, β-glucan digestibility values at the ileum and for the total tract were 84-85 and 100%, respectively for pigs fed a barley-based (74%) diet (Fadel et al., 1988). According to Inborr (1993), β-glucan digestibility in the fourth quarter of the small intestine of early-weaned pigs was 71 and 32% with and without BGase addition to a barley (34.8%)/wheat (34.8%)-based diet. The lower digestibility of β-glucan in early-weaned pigs is possibility associated with less microbial enzyme activity in the upper digestive tract due to the young age of the animals (Bach Knudsen et al., 1993). Although there is considerable variation of ileal β-glucan digestibility in pigs, total tract digestibility is 100% in most experiments even without the addition of BGase into the diets. Beta-glucan is not digested by the host animal but as indicated above digestibility studies, can be fermented by bacteria in the digestive tract. Bacterial fermentation in the lower GIT results in the production of volatile fatty acids (VFA) including acetic, propionic, butyric and lactic acid. Beta-glucan fermentation has also been reported in the crop of broiler chickens. Acetate concentration in the crop increased with the addition of endo-1,3(4)-β-glucanase (100 U/g) and endo-1,4-β-xylanase (300 IU/g) to a barley-based diet (57.3% in starter and 61% in grower) in broiler chickens (Józefiak et al., 2005). In the same study, the caecal propionic acid and total VFA values also increased with the addition of the enzyme. Increased fermentation products were also reported in broiler chickens fed barley- and oat-based diets with the addition of endo-1,3(4)-β-glucanase (100 U/g) and endo-1,4-β-xylanase (300 U/g) (Józefiak et al., 2006). The differences in VFA have also been reported in pigs fed cereal β-glucan. Acetic acid to propionic acid ratio in the caeca, propionic and valeric acid in the proximal colon increased with the addition of endo-1,3(4)-β-glucanase and endo-1,4-β-xylanase to barley- and oat-based diets in finisher pigs (O'Shea et al., 2010).

2.2.3. Effects of dietary beta-glucan

Barley β-glucan can negatively affect poultry in terms of production performance, digestive physiology, and gastro-intestinal health. Soluble β-glucan increases digesta viscosity of broiler chickens (Classen et al., 1985; Salih et al., 1991; Friesen et al., 1992; Rodríguez et al., 2012), and thereby negatively affects digestibility of nutrients including fat, protein and starch (Edney et al., 1989; Friesen et al., 1992; Fuente et al., 1995; Rodríguez et al., 2012). Low nutrient digestibility is thought to be the result of the reduced interaction of digestive enzyme and substrate, rates of nutrient diffusion and feed passage rate in chickens (Johnson and Gee, 1981; Fengler and Marquardt, 1988). Further, reduced feed passage rate leads to less feed intake in broiler chickens (Mathlouthi et al., 2002a). Soluble fibre including β-glucan, increases viscosity in the digestive tract resulting in decreased nutrient digestibility; reduction in nutrient digestibility increases digestive tract size. The gizzard size increases as a result of increased retention time in birds fed HB (Brenes et al., 1993a), which is a direct effect of fibre. However, increased weight and length of other digestive tract sections might be associated with a physiological response to undigested nutrients or short chain fatty acids (SCFA). These constituents in the digestive tract activate nutrient sensing L-cells in the small intestine, and release hormones including protein YY (PYY), glucagon-like peptide (GLP) 1 and 2 (Hiramatsu et al., 2005; Aoki et al., 2017). The digestive tract size is increased by GLP-2 (Ghatei et al., 2001), and PYY and GLP-1 reduce gastric emptying and increase satiety (Taylor, 1993; Zhou et al., 2008). Miyamoto et al. (2018) found high dietary β-glucan increased the secretion of PYY and GLP-1 in conventional but not in germfree mice, and it signifies the involvement of fibre fermentation and SCFA in the release of hormones.

Beta-glucan in cereals also affects gut health in chickens by enhancing the proliferation of bacteria in the small intestine of chickens due to high digesta viscosity and lower feed passage rate (Kaldhusal and Hofshagen, 1992; Józefiak et al., 2006). Bacterial modification in the chicken digestive tract affects nutrient digestibility especially the digestion of fats due to bile-acid deconjugation (Campbell et al., 1983; Feighner and Dashkevicz, 1988). In addition, it also increases the susceptibility to enteric diseases including necrotic enteritis due to the viscous fibre induced colonization of pathogenic bacteria in the digestive tract of chickens (Annett et al., 2002;

Timbermont et al., 2011). A higher level of soluble β-glucan in the diet causes excretion of sticky droppings that increase the wetness of the litter, and results in foot pad dermatitis, hock burns and breast blisters in broiler chickens, and egg soiling (McNab and Smithard, 1992). Insoluble β-glucan present in the cereal cell wall can also impact nutrient digestibility due to the encapsulation of nutrients including starch and protein and thereby reducing access to the digestive enzymes (Hesselman and Aman, 1986).

Cereal β-glucan is also considered as an anti-nutrient factor in pigs since it can negatively affect pig production. The anti-nutrient effects are associated with high digesta viscosity (Hooda et al., 2011), which leads to poor nutrient digestibility and performance (Mitchall et al., 1976; Graham et al., 1989). However, the degree of viscosity and performance responses in pigs is inconsistent and small compared to poultry. The lower response on the digesta viscosity in pigs is attributed to the high levels of β-glucan digestibility and endogenous secretions-associated high moisture conditions in the small intestine of pigs (Fadel et al., 1988; Bach Knudsen and Hansen, 1991; Inborr, 1993).

2.3. Antibiotic-free poultry production

Antibiotics have been added to poultry diets to maintain health and production efficiency for the last few decades (Engberg et al., 2000). Antibiotics are mostly applied to prevent enteric infections including necrotic enteritis, colibacillosis and salmonellosis in poultry. The type of antibiotic use varies from country to country due to the differences in animal husbandry, disease prevalence and economy. However, the common antibiotics which were used in poultry around the world includes polymyxins, penicillins, tetracyclines, sulfonamides and trimethoprim (Roth et al., 2019). The use of specific antibiotics in poultry feed has decreased voluntarily or due to legislation, in response to the emergence of pathogenic bacterial resistance in animals and humans, which impacts public health and food safety (Diarra et al., 2007; Garcia-Migura et al., 2014; Roth et al., 2019). The common bacterial species in poultry that are resistant to specific antibiotics comprises *Escherichia coli, Campylobacter jejuni, Campylobacter coli, Enterococcus faecium, Enterococcus faecalis* and *Salmonella enterica* (Garcia-Migura et al., 2014).

2.4. Alternatives to antibiotics

Investigation of alternatives to in-feed antibiotics is now a major focus due to the limited access to antibiotics for use in poultry feeds to prevent enteric disease and enhance performance (Diarra and Malouin, 2014). Feed additives including prebiotics, probiotics, symbiotics, organic acids and essential oils are the main focus of nutritional control of gut health in poultry (Huyghebaert et al., 2011; Ducatelle et al., 2015; Gadde et al., 2017). However, the role of dietary components on gut health is also an emerging subject as an alternative approach to the use of prophylactic antibiotics.

2.4.1. Prebiotics

Prebiotic is "a substrate that is selectively utilized by host microorganisms conferring a health benefit" according to the International Scientific Association of Probiotics and Prebiotics (Gibson et al., 2017). Fermentable carbohydrates including xylo-oligosaccharides (XOS), arabinoxylo-oligosaccharides (AXOS), fructo-oligosaccharides (FOS), inulin and mannan-oligosaccharides (MOS) act as prebiotics in poultry feed. The mechanisms involved with prebiotics include provision of nutrients to the host and gut microbiota, prevention of pathogen adhesion to host intestinal epithelial cells, interaction with the host immune system, and modification of gut morphological structure by modulating intestinal bacteria (Pourabedin and Zhao, 2015). They are advantageous over probiotics as they selectively stimulate beneficial bacteria that are naturally present in the intestinal environment (Roberts et al., 2015).

Carbohydrate fermentation refers to anaerobic bacteria degradation of undigested carbohydrates into short chain fatty acids (SCFA) such as acetic, propionic and butyric acid (Choi et al., 2015; Józefiak et al., 2004). The caeca are the primary site of fermentation in poultry. Short chain fatty acids are absorbed into the systemic circulation through the caecal epithelial membrane and are used as an energy source by the enterocytes in poultry (Annison et al., 1968), although the energy derived from SCFA is relatively low, contributing 3-5% of the total energy requirements of chickens (Choct et al., 1992; Jørgensen et al., 1996). In addition, SCFA reduce intestinal pH, and inhibit the growth of some pathogenic bacteria by dissipating the proton motive force across the bacterial cell membrane (Russell, 1992). Further, SCFA have toxic and bacteriostatic effects towards some pathogenic bacteria but do not inhibit beneficial

gastro-intestinal bacteria (McHan and Shotts, 1993; Van der Wielen et al., 2000). It also improves mineral absorption in the host, and the growth of intestinal epithelial cells (Choi et al., 2015). In general, all types of SCFA cause enhancement of intestinal mucosal blood supply, activation of mucin release, generation of an acidic environment in gut and stimulation of electrolyte and water absorption (Plöger et al., 2012). The presence of fermentable carbohydrate shifts microbial proteolytic fermentation into more carbohydrate fermentation as caecal microbes prefer carbohydrate as their main energy source. Dietary fermentable carbohydrate in chickens reduces hind gut protein fermentation, improves gut health and increases growth performance (Qaisrani et al., 2015).

2.4.1.1. Mannan-oligosaccharides (MOS)

Mannan-oligosaccharides are mannose oligomers linked by β-1,4 glycosidic bonds. They are present in yeast (*Saccharomyces cerevisiae*) cell wall and some plants. Mannan-oligosaccharides are not digested in the small intestine because chickens do not have the enzymes to digest MOS, and they reach lower GIT undigested (Pourabedin and Zhao, 2015). Feeding MOS to chickens has been shown to modify the lower GI microbial population, specifically reducing pathogenic bacteria (*Clostridium perfringens, Salmonella, Campylobacter* and pathogenic *E.coli*) and increasing the number of beneficial bacteria (Lactobacilli and Bifidobacteria) and bacterial diversity (Ofek and Beachey, 1978; Baurhoo et al., 2007; Kim et al., 2011; Yitbarek et al., 2012; Pourabedin et al., 2014; Corrigan et al., 2015). This effect has been attributed to the availability of more fermentable carbohydrates. Further, Mannan-oligosaccharides reduce the binding of gram-negative bacteria, including *Salmonella* colonization in chickens, onto intestinal epithelial cells through competitive binding of MOS with mannose-specific lectin (FimH) of type 1 fimbriae (Ofek and Beachey, 1978). The intestinal morphology of chickens was also affected by the supplementation of MOS in poultry diets. Mannan-oligosaccharides increased the villus height, goblet cell density and mucin secretion (MUC2 gene expression and intensity of sulpho-mucins) in the small intestine of broilers (Baurhoo et al., 2007; 2009; Chee et al., 2010; Pourabedin et al., 2014) indicating improved intestinal epithelial integrity and immune defense, which is beneficial in terms of poultry gut health. In addition, the supplementation of MOS into the poultry diets triggered a pro-

inflammatory response against intestinal pathogens, by upregulating genes related to toll-like receptors (TLR-2b and TLR4) and pro-inflammatory cytokines (IFN-γ and IL12) in broilers challenged with *Cl. Perfringens.* However, MOS terminated the inflammatory response in chickens during chronic and systemic inflammation, according to the reduced gene expression related to cytokines and glucose mobilization (Yitbarek et al., 2012).

2.4.1.2. Fructo-oligosaccharides (FOS) and inulin

Fructo-oligosaccharides are linear polymers of β-(2-1)-linked fructose units (DP ≤ 10), terminated by a glucose molecule. They are naturally occurring oligosaccharides that have a plant origin; a longer version of FOS (DP > 10) is called inulin. Fructo-oligosaccharides are not digested in the small intestine of poultry digestive enzymes, and therefore reach the lower GIT and undergo microbial fermentation (Pourabedin and Zhao, 2015). Fermentation of FOS leads to the modification of digestive tract microbiota, and thereby indirectly affects immune function and gut morphology in chickens (Xu et al., 2003; Shang et al., 2015). However, direct effects of FOS on the immune system has also been reported, and it is via the interaction with carbohydrate receptors located on intestinal epithelial cells and immune cells (Seifert and Watzl, 2007). *In vivo* and *in vitro* studies have shown FOS/inulin can modify the microbial population of the chicken GIT. Fructo-oligosaccharides/inulin specifically increased Bifidobacterium and Lactobacillus numbers, while decreasing *Clostridium perfringens*, pathogenic *Escherichia coli* and *Salmonella* Enteritidis in chickens (Xu et al., 2003, Rebole et al., 2010; Kim et al., 2011; Babu et al., 2012; Zhao et al., 2013). In addition, dietary fructans (average DP of 10) have been shown to decrease ammonia concentration in the caeca and increase caecal butyric and lactic acid concentrations in chickens (Rebole et al., 2010; Zhao et al., 2013), indicating the shift of microbial fermentation towards saccharolytic fermentation, and a potential improvement of GI barrier function. It has also been observed that dietary inulin/FOS improves intestinal immune function in broiler chickens. Inulin decreased pro-inflammatory cytokines (IL1β, IL6 and IFNγ) and CD4+ T lymphocytes, while increasing IgA concentration and mucin gene expression, with the effect more obvious in young than older broiler chickens (Babu et al., 2012; Huang et al., 2015). Further, feeding FOS decreased heterophil and increased monocyte counts in blood (Shang et al., 2015). Feeding FOS also improved the intestinal morphology of chickens by increasing villus

and microvillus height, villus height: crypt depth and decreasing crypt depth (Xu et al., 2003; Shang et al., 2015). Furthermore, broiler production performance and digestive enzyme activity also increased with the addition of FOS to the diets (Xu et al., 2003). It has been observed that dietary addition of 5-10 g/kg FOS over the control and higher doses of FOS is beneficial in terms of improving immune function in young broiler chickens according to cytokine production, T lymphocyte ratios and gene expression of immunoglobulin levels in the digestive tract (Huang et al., 2015). Further, Xu et al. (2003) demonstrated beneficial effects on the digestive tract morphology and microbiota with the dietary addition of 4 g/kg FOS in an experiment used 0, 2, 4 and 8 g/kg FOS levels. Therefore, the dose response of prebiotics is important when determining its' response on broiler chickens.

2.4.1.3. Arabinoxylo-oligosaccharides (AXOS)/ xylo-oligosaccharides (XOS)

Arabinoxylo-oligosaccharides and xylo-oligosaccharides are the chains of β-1,4-linked D-xylopyranoside units that originate from depolymerization of arabinoxylan (AX) found in cereal grains including wheat, rye, corn and barley. Xylose is not digested because chickens lack the enzymes capable of hydrolyzing β-glycosidic linkages, and therefore reach the caeca and colon (Pourabedin and Zhao, 2015). The dietary addition of XOS/AXOS and soluble AXOS increased the level of caecal bifidobacteria in broilers compared to the birds fed wheat-based control diets (Courtin et al., 2008a; b). In addition, dietary XOS increased the number of lactobacilli, *Clostridium* cluster XIV in the caeca and *Roseburia* in the caeca of broiler chickens (De Maesschalck et al., 2015; Pourabedin et al., 2015; 2017). The supplementation of XOS/wheat bran AXOS resulted in the reduction of *Salmonella* shedding, colonization in the caeca, and the spleen translocation of *Salmonella* Enteritidis challenged broiler chickens (Eeckhaut et al., 2008; Pourabedin et al., 2017). Further, XOS increased butyril-coenzyme A synthesis genes in the caeca (De Maesschalck et al., 2015), indicating possible increased capacity for butyric acid production in chicken caeca. This finding is confirmed by the increased butyric acid concentration in the caeca of broiler chickens fed diets containing XOS (Ding et al., 2018; Yuan et al., 2018). Furthermore, increased total SCFA, acetic and propionic acids were also reported with the addition of XOS to broiler diets (Pourabedin et al., 2015; Yuan et al., 2018). Moreover, intestinal epithelial morphology has been positively modified by diet XOS in broiler

chickens. Feeding XOS increased villi height and villi height to crypt depth ratio in the small intestine of chickens (De Maesschalck et al., 2015; Ding et al., 2018). Xylo-oligosaccharides also down-regulated the expression of genes related to the pro-inflammatory response including IFNγ, TLR5 and LPS induced TNFα in the chicken small intestine (Yuan et al., 2018). In addition, broiler production performance was also increased with the dietary addition of AXOS (Keerquin et al., 2017). The dose-dependent response of XOS has been reported in broiler chickens and laying hens. Xylo-oligosaccharides increased the health and immune function of laying hens since the expression of immune genes, and the caecal bacterial counts and fermentation metabolites increased linearly when the diets consisted of 0 to 0.05% XOS; the level increasing with 0.01% (Ding et al., 2018). Further, dietary addition of 2 g/kg over 0 and 1 g/kg XOS demonstrated higher carbohydrate fermentation and beneficial bacterial counts in the caeca of broiler chickens (Pourabedin et al., 2015).

Xylo-oligosaccharides and AXOS exert their prebiotic effects through increased microbial fermentation according to the literature (Masey-O'neill et al., 2014; De Maesschalck et al., 2015; Lee et al., 2017). The degree of AXOS fermentation is related to the molecular weight reduction of arabinoxylan in the digestive tract through solubilization and enzyme hydrolysis (Bautil et al., 2019). However, the fermentation mechanism has been questioned in recent papers, and suggests the low doses of XOS used in the previous studies is not sufficient to produce a measurable effect on broiler production even it is totally converted to SCFA (Ribeiro et al., 2018). Therefore, the suggested concept was XOS act as signaling molecules for gut microbiota to utilize fibre more effectively and increase the digestion efficiency of nutrients (Bedford et al., 2018; Ribeiro et al., 2018; Bautil et al., 2019).

2.4.1.4. Beta-glucan

The fermentation of cereal β-glucan has been investigated in many studies in a wide range of species (Józefiak et al., 2005; 2006; Queenan et al., 2007; Shen et al., 2012; Metzler-Zebeli and Zebeli, 2013). However, effect of cereal β-glucan on fermentation and other physiological parameters was minimal and inconsistent in chickens. Further, molecular weight distribution of cereal β-glucan has not been investigated in relation to the digestive tract of chickens. The addition of 1 g/kg Avizyme (100 U/g endo-1,3(4)-β-glucanase and 300 U/g endo-

1,4-β-xylanase) to a barley-based diet (57% in starter, 61% in grower) increased acetic acid concentration in the crop, and propionic acid and total SCFA in the caeca of broiler chickens, which indicates enhanced carbohydrate fermentation due to BGase supplementation (Józefiak et al., 2005). According to the research done by Józefiak et al., (2006) 1 g/kg Avizyme (100 U/g endo-1,3(4)-β-glucanase and 300 U/g endo-1,4-β-xylanase) to a barley-based diet (66% in starter, 70% in grower) only increased lactic acid concentration and decreased pH in the crop content of broiler chickens, but not in the other digestive tract locations including the ileum and caeca, indicating less enzyme effects on carbohydrate fermentation. Further, exogenous enzyme supplementation (Avizyme containing 100 U/g endo-1,3(4)-β-glucanase and 300 U/g endo-1,4-β-xylanase) decreased potentially pathogenic Enterobacteriaceae population while increasing beneficial *Bifidobacterium* spp in the caeca of broiler chickens when given a barley (66% starter, 70% grower) and rye-based (62% starter, 65% grower) diets (Józefiak et al., 2010). The previous research shows the effect of cereal β-glucan on microbial fermentation and other physiological parameters was minimum and inconsistent in chickens.

The experiments used feed BGase to depolymerize β-glucan into oligosaccharides. However, the degree of polymerization of β-glucan (or β-glucan molecular weight) and the exact number of oligosaccharides have not been assessed in the digestive tract contents. Further, previous studies did not differentiate the effects of endoxylanase and BGase since the enzymes had similar enzyme activity, except in a few production and viscosity studies in chicken (Mathlouthi et al., 2002b; Dos Santos et al., 2013). Moreover, the clarification of the number of units of each enzyme does not confirm enzyme purity in the diets. Therefore, it warrants the investigation of pure enzyme effect, and the enzyme dosage effect on the responses in chickens.

2.5. Feed enzymes

Enzymes are biological catalysts capable of accelerating chemical reactions. They are proteins with a complex molecular structure and substrate-specificity. Moisture, temperature, pH, enzyme and substrate concentrations are important factors for enzyme activity (Ravindran, 2013). Exogenous enzymes are used for different reasons in poultry industry such as breakdown of anti-nutritional factors in feed, increase availability of nutrients enclosed within cell walls of cereals, breakdown specific chemical bonds that are not broken-down by endogenous enzyme of

poultry, supplement enzymes to young animals that have immature digestive system and reduce variability in nutritive value of feedstuffs (Munir and Maqsood, 2013). The most common enzymes that are commonly used in the feed industry include phytase, xylanase, β-glucanase and protease. The most used enzyme in the monogastric animal feed is phytase which increases digestibility of phosphorous, calcium, amino acids, and energy by reducing the anti-nutritional effect of phytate (Dersjant-Li et al., 2014). Xylanase is used in animal feed (mostly wheat-based diets) to reduce the negative effects of soluble arabinoxylan including high digesta viscosity that affects nutrient digestibility and gut microbiota colonization (Bedford, 2018). Beta-glucanase is a non-starch polysaccharidase used in barley- and oat-based diets to minimize the soluble β-glucan derived high digesta viscosity (Józefiak et al., 2005; 2006; Clarke et al., 2018). Proteases are used in animal feed to improve protein digestibility of feed ingredients, and thereby reduce the available proteins as the substrates for bacterial fermentation which is harmful in monogastric animals (Philipps-Wiemann, 2018). However, only BGase is covered in detail due to its' importance on the current research.

2.5.1. Beta-glucanase

Beta-glucanase is a non-starch polysaccharide degrading enzyme that commonly used in the feed industry when poultry is fed with barley- or oats-based diets since it results in a more consistent and positive response in poultry in terms of performance and GI physiology. Beta-glucanase is an endo-enzyme that hydrolyzes β-(1-4) linkages which are located next to β-(1-3) linkages (Kuge et al., 2015). Cellulase also consists of the same enzyme activity however, it hydrolyzes β-(1-4) linkages despite the location and has the possibility of resulting glucose monomers (Wang et al., 2011). The purpose of BGase supplementation is to reduce the negative effects of β-glucan, specifically reducing digesta viscosity and nutrient encapsulation that negatively affect nutrient digestibility and the gut microbial population of monogastric animals. However, many previous studies failed to demonstrate the individual effect of BGase, since the feed enzymes were consisted of similarly high enzyme activity of both BGase and xylanase, although the responses were mentioned as pure effects of BGase. Beta-glucanase reduces digesta viscosity (Salih et al., 1991; Fuente et al., 1995; Yu et al., 1998; Józefiak et al., 2005; 2006), increases nutrient digestibility (Edney et al., 1989; Brenes et al., 1993a; Pertilla et al., 2001;

Ravindran et al., 2007) and diet apparent metabolizable energy (Potter et al., 1965; Fuente et al., 1995; Pertilla et al., 2001; Ravindran et al., 2007), and increases production performance (Classen et al., 1988a; Campbell et al., 1989; Salih et al., 1991; Almirall and Esteve-Garcia, 1995; Józefiak et al., 2005; 2006) in broilers fed barley- or oat-based diets. The concomitant use of BGase and xylanase in feed improved production performance and nutrient digestibility in poultry and swine fed wheat- and barley-based diets through depolymerizing non-starch polysaccharides, and thereby reducing digesta viscosity and increasing fibre digestibility (Mathlouthi et al., 2002a; Owusu-Asiedu et al., 2010; Munyaka et al., 2015; Clarke et al., 2018) since both grains contain arabinoxylan in addition to β-glucan as NSP. Very few research studies have attempted to differentiate the effects of BGase and xylanase when feeding barley- and oat-based diets. The individual and combined effects of BGase and xylanase have been studied by Dos Santos et al. (2013), demonstrating no improvement in the performance of broiler chickens with individual or combination of enzymes in barley-based diets. However, the digesta viscosity reduced with xylanase and, more reduction with BGase. Further, the enzyme combination resulted in the lowest viscosity in both *in vitro* and *in vivo* studies (Mathlouthi et al., 2002b; Dos Santos et al., 2013).

Beta-glucanase addition to swine diets has been positive, but less consistent than in chickens. In some studies BGase resulted in increased nutrient digestibility and performance of pigs (Baidoo et al., 1998; Bedford et al., 1992; Clarke et al., 2018). However, in other swine research, BGase slightly improved the nutrient digestibility and performance, and with no improvement in some instances (Graham et al., 1989; Inborr et al., 1993; Thacker et al., 1992). Again, the high digestibility of β-glucan in the small intestine of pigs is consistent with these results.

2.6. Gastro-intestinal (GI) health and barrier function

Gut health refers to the maintenance of healthy microbiota in the GI tract by establishing beneficial bacteria and inhibiting colonization of harmful/pathogenic bacteria. The composition of microbiota in the chicken gut depends on the age and type of chicken, dietary factors and geographical location that the birds raised. Beneficial bacteria facilitate gut health by stimulating the immune system through non-pathogenic mechanisms, inhibiting growth/establishment of

harmful microbes in GIT and producing vitamins. Harmful bacteria down-regulate gut health by causing localized/systemic infections, intestinal putrefaction and toxin formation (Yegani and Korver, 2008).

Gastro-intestinal barrier function is associated with gut health as it minimizes and/or prevents pathogenic organisms from becoming systemic via translocation through the digestive tract epithelium. Barrier function in chickens is mainly established by tight junctions, mucus secretion, GI tract lumen pH and inflammatory reactions associated with immune cells.

2.6.1. Intestinal epithelial cells and tight junctions

Tight junctions (TJ), adherent junctions, gap junctions and desmosomes are the main cell to cell junctions in vertebrates, and TJ play a major role in maintaining GI barrier function against luminal microbes (Feldman et al., 2005). The epithelial membrane of the digestive tract functions as a physical barrier between luminal microbes and immune cells in lamina propria and submucosa of the gut wall. The epithelial cells are maintained by specialized intercellular structures namely TJ and adherent junctions. These intercellular junctions also connect to the actin cytoskeleton, provide mechanical support to the junctions and play a role in the remodeling of TJ and adherent junctions (Chen et al., 2006). Tight junctions seal adjacent epithelial cells control the passage of ions, water and other molecules between cells and maintain epithelial cell polarity (Feldman et al., 2005). Tight junctions contain several transmembrane proteins including occludin and junctional adhesion molecules.

Many studies have demonstrated that the pathogenic bacteria-induced disintegration of TJ in chickens and impairment of the GI barrier (Förster, 2008; Chen et al., 2015). As an example, *Campylobacter jejuni* infection disintegrates GI barrier function. *Campylobacter jejuni* adheres to and invades intestinal epithelial cells. Further, intracellular *C. jejuni* causes loss of trans-epithelial resistance and occludin redistribution and opens the TJ. The latter effect facilitates *C. jejuni* interaction with host cells, and results in uncontrolled inflammation, shown by increased activation of NF-κB factor and IL8 expression (Chen et al., 2006). Ye et al. (2006) observed TNF-α induced mechanism which increases intestinal TJ permeability, since TNF-α activates NF-κB which binds with the Myosin light-chain kinase (MLCK) promoter region, increases

MLCK expression and results in the opening of the intestinal epithelial TJ barrier. Salmonellosis also causes TJ disruption in chickens. Jepson et al. (2000) demonstrated infection of MLCK epithelial layers due to the colonization of *Salmonella* Typhimurium and it is accompanied by increased TJ permeability and the contraction of perijunctional actinomycin.

2.6.2. Mucus

The mucus layer is the first line of innate immune defence, which foreign bacteria and other pathogens in the intestinal lumen encounter, when trying to go through the intestinal mucosa to reach the systemic circulation. The mucus layer is formed by mucin glycoproteins that are secreted by goblet cells which are specialized columnar epithelial cells. Mucins are high molecular weight, highly glycosylated glycoproteins (Forder et al., 2007), and act as a structural component of the intestine. They are involved in protection, lubrication and transport between the lumen and intestinal epithelium (Deplancke and Gaskins, 2001). Mucin has binding sites for both commensal and pathogenic organisms, and performs a defensive role (Forder et al., 2007). Mucin glycoproteins can be divided into three categories according to histochemical staining, and they are namely neutral, sialic acid-containing and ester sulphate-containing mucins. Both sialic acid and sulphate-containing mucins have properties of intestinal acidic mucin. They are distributed throughout the intestinal epithelium and dominate in the large intestine, whereas neutral mucin predominates in the gastric mucosa (Deplancke and Gaskins, 2001). Sulphate mucins are usually higher in immature goblet cells (Fontaine et al., 1996). Fontaine et al. (1996) observed small intestine goblet cell mucus composition was affected by gut microbiota at 3 to 4 days post-hatch. The total number of goblet cells containing acidic mucins was not affected by bacterial colonization in the intestines. However, sulphated mucin content was reduced and sialylated mucin content was increased due to bacterial colonization in chickens.

Intestinal microbes affect goblet cell dynamics directly by local release of bioactive factors or indirectly by activation of host immune cells. Mucus is advantageous to both commensal and pathogenic bacteria, and both types of bacteria have the ability to regulate mucus synthesis from the intestinal goblet cells (Deplancke and Gaskins, 2001). However, overproduction of mucus is associated with inflammation caused by a bacterial infection (Smirnova et al., 2003). Some bacteria have mucin specific glycosidases and proteases, which

can degrade mucus and thereby facilitate bacterial colonization in the GIT. Bacterial endotoxins (eg: LPS in gram-negative bacteria cell wall) upregulate mRNA expression and secretion of pro-inflammatory cytokine IL-8 and mucin genes. In addition, microbial fermentation end-products including SCFA induce mucin secretion in the intestine. Cytokines increase the extent of mucin production and goblet cell proliferation, and modify the glycosylation of mucins (Fontaine et al., 1996). According to the findings of Dohrman et al. (1998), both gram-positive and gram-negative bacteria upregulate mucin gene expression in the epithelial cells.

2.6.3. Inflammation and cytokine production

Inflammation is a localized physical condition that is protective and occurs in response to an injury or infection. Erythaema, edaema, pain, high temperature and loss of function are symptoms associated with inflammation (Takeuchi and Akira, 2010). Cytokines are regulators of both innate and acquired immunity and are produced as a result of inflammation (Zhang and An, 2007). In addition, cytokines regulate epithelial permeability and ion transport directly or indirectly, which ultimately influences gut health. However, a drastic increase of cytokines during an inflammatory reaction is associated with the unregulated production of pro-inflammatory cytokines or inadequate synthesis of anti-inflammatory cytokines (McKay and Baird, 1999).

Cytokines play a major role in the control of infectious diseases in poultry. Cytokines are classified into four groups based on their function, and they are namely pro-inflammatory cytokines, Th1, Th2 and Th3 cytokines. Pro-inflammatory cytokines mediate inflammation during disease or injury, and they include IL-1β, IL-6, IL-8 and chemokines (Wigley and Kaiser, 2003). It has been shown the mRNA expression of IL-1β in the digestive tract increased following both *E. tenella* and *E. maxima* infections in chickens (Laurent et al., 2001). Lynagh et al., (2000) demonstrated the production of IL-6 during *E. tenella* infection in chickens. In addition, increased IL-6 mRNA expression was observed in chicken cells invaded with *Salmonella* Typhimurium or Enteritidis (Kaiser et al., 2000). An increased level of IL-8/chemotactic and angiogenic factor was observed in chicken cells invaded by *S.* Typhimurium (Wigley and Kaiser, 2003). Th1 cytokines include IL-2 and IFN-γ and are involved in the activation of macrophages and the development of cell-mediated immunity. A high level of IL-2

mRNA expression has been seen in the digestive tract of chickens infected with *Eimeria* (Choi and Lillehoj, 2000). Th2 cytokines are responsible for the development of the humoral response. Th3 cells produce Th3 cytokines including transforming growth factor-β (TGF-β), and they are involved in the development of the mucosal immune response (Wigley and Kaiser, 2003). Choi et al, (1999) observed a high level of TGF-β_4 mRNA expression in the caecal tonsils, spleen and duodenum following *E. acervulina* infection in chickens.

The innate immune system provides an immediate response against pathogens. Toll-like receptors (TLR), a type of pattern recognition receptor (PRR) specifically bind with microbial pathogens, and TLR respond to pathogen-associated molecular patterns (PAMP) shared by many pathogens. Toll-like receptors cause activation of NF-κB and secretion of pro-inflammatory cytokines as a protective immune response to an infection (Abreu, 2003). The NF-κB pathway regulates pro-inflammatory cytokine production and leukocyte recruitment, which are important for inflammation (Lawrence, 2009). Hong et al. (2006) observed an up to 2000-fold increase in transcripts of pro-inflammatory cytokines (IFN-α, IL-1β, IL-6, IL-17) following a primary infection of *E. acervulina* and *E. tenella* in chickens. However, there were no changes in cytokine gene expression following a secondary *Eimeria* infection. Increasing transcripts encoding Th1 regulatory cytokines (IFN-γ, IL-2, IL-10, IL-12, IL-15, IL-16 and IL-18) were found following primary infection of *E. acervulina* infection. However, *E. tenella* primary infection resulted in up- regulation, down-regulation, or no change in gene expression for different cytokines.

2.6.4. Antimicrobial peptides

Antimicrobial peptides are an essential element of the innate immune system. Chickens have three types of antimicrobial peptides including β-defensins (Gallinacins), cathelicidins and LEAP-2 (Lynn et al., 2004). There are 14 avian β-defensins (AvBD 1 - 14) that have been identified in chickens (van Dijk et al., 2008). Antimicrobial peptides are secreted by intestinal epithelial cells and paneth cells located at the base of intestinal crypt cells. The main functions associated with antimicrobial peptides are differentiation, activation and chemotaxis of leukocytes, increased phagocytosis, increased pro-inflammatory cytokine secretion and also anti-inflammatory cytokine production to inhibit LPS induced inflammation and wound healing.

Most AvBDs show anti-bacterial effects against bacteria and fungi by direct killing (Cuperus et al., 2013). Gene expression of AvBD changes during infectious diseases of chickens including coccidiosis, and the challenge with *E. acervulina* down-regulated the expression of AvBD 1, 6, 10, 11, 12 and 13 in the duodenum (Su et al., 2017).

2.6.5. Gastro-intestinal epithelial cell growth/proliferation

The intestinal epithelial cell layer functions as one of the main components of the GI barrier. It prevents entering of pathogenic organisms, toxins and other antigens from the intestinal lumen into the circulation (Williams et al., 2015). The stem cells in the intestinal crypt cells of mammals develop into enterocytes, migrate along the villus, and are shed into the intestinal lumen at the villus tip during the cell proliferation process (Chang and Leblond, 1974). However in chickens, cell proliferation has also been observed in villi epithelial cells, but lower than in the crypt cells. (Uni et al., 1998). The balance between the rate of apoptosis of intestinal epithelial cells in villi and generation of new epithelial cells in crypts is important to maintain a healthy GI tract function in animals (Williams et al., 2015).

2.6.6. Gastro-intestinal microbial population

The GI microbiota plays an important role in the GI barrier of chickens. Commensal gut microbiota assists with nutrient digestion and gut mucosal proliferation. The commensal bacteria ferment non-digestible nutrients and produce SCFA and lactic acid as fermentation products (Lan et al., 2005); fermentation products improve GI barrier function by increasing mucosal blood supply, epithelial cell growth, water and electrolyte absorption, mucus secretion, tight junction protein expression, immune modulation by cytokine production and providing energy to the epithelial cells of the host (Plöger et al., 2012). Microbiota in the caeca synthesize amino acids from non-protein nitrogen that originates from urinary nitrogen as a result of anti-peristaltic movement of digesta from the cloaca. The presence of GI bacteria also increases digestive enzyme activity. Mucosal alkaline phosphatase activity increased in conventional birds compare to GF chicks in the study of Palmer and Rolls (1983). Commensal GI microbiota has an important role in preventing pathogenic bacterial colonization through competitive exclusion. They resist pathogenic bacterial colonization by competing for the nutrients and attachment sites of the intestinal mucosa and by producing bacteriocins (Lan et al., 2005).

2.7. Common gastro-intestinal diseases in chickens

Digestive tract diseases in chickens that reduce production efficiency include coccidiosis, necrotic enteritis, salmonellosis and campylobacteriosis. The latter two diseases are also associated with zoonotic disease risk (Schlundt et al., 2004; Smith et al., 2019). Therefore, it is important to investigate control methods for these diseases as alternatives to the use of in-feed antibiotics in chickens.

2.7.1. Coccidiosis

Coccidiosis is a disease caused by protozoan parasites of the genus *Eimeria.* It is an economically important, widely reported parasitic disease in poultry (Tan et al., 2014b). It results in annual global economic losses of about 3 USD billion, including production losses and disease prevention and treatment costs (Blake and Tomley, 2014). The disease can cause severe mortality and poor performance in chickens.

There are seven species of *Eimeria* (*E. acervulina, E. brunetti, E. maxima, E. mitis, E. necatrix, E. praecox* and *E. tenella*) capable of infecting chickens (De Gusseum, 2007). Coccidiosis is usually caused by the association of two or more species of *Eimeria* in chickens (Paiva and McElroy, 2014). Each species of *Eimeria* has a predilection site in the GIT. *E. acervulina* develops in the duodenum, *E. maxima* and *E. mitis* develop in the middle part of the SI, *E. tenella* develops in the caeca, *E. brunette* develops in the caeca and the rectum, and *E. necatrix* develops in the SI (Raman et al., 2011). Further, one particular species of *Eimeria*, which infects chicken was not able to produce the disease in another avian host, indicating host specificity of *Eimeria* (Johnson, 1923).

2.7.1.1. The life cycle of Eimeria

Eimeria species have a complex life cycle (Figure 2.1), consisting of two developmental stages called exogenous and endogenous. The process called sporogony occurs in the exogenous stage of the life cycle (Blake and Tomley, 2014). Price (2012) described the life cycle of *Eimeria* as follows. Unsporulated oocysts (non-infective) are released to the environment via faeces of birds during the exogenous stage. These unsporulated oocysts undergo sporulation after around 48 h at the optimal temperature (30^0C), relative humidity and oxygen level. The sporulated

oocyst contains 4 sporocysts and each of these contains two infective sporozoites. Infective sporozoites can survive in the litter for a long time and transmit to birds by the faecal-oral route. The endogenous developmental stage occurs inside the intestine of the host and starts with the ingestion of sporulated oocysts by the bird. The grinding action of the gizzard and the digestive enzymes released from upper SI facilitate release of sporocysts from the oocyst, and then sporozoites from the sporocyst. Sporozoites directly penetrate the epithelial cells of the host intestine in various regions of the digestive tract depending on the species of *Eimeria*. The sporozoite develops into a trophozoyte inside the host cell, and then divides asexually to form many merozoites, and this process is called merogeny. Merozoites invade new intestinal epithelial cells to complete another cycle of merogony, and the number of merogony cycles (2-4) can vary depending on the *Eimeria* species. Merozoites then invade new intestinal epithelial cells, and start the process of gametogony, which is the sexual phase of the life cycle. In this phase, parasites develop into macrogametes and microgametes, and form a zygote, which later develops into an unsporulated oocyst. The unsporulated oocyst comes out of the intestinal epithelial cell, and shed in the faeces, thereby completing the *Eimeria* life cycle. Other than the above-mentioned intestinal phases of the life cycle, there is an extra-intestinal phase of *Eimeria*. *Eimeria* sporozoite migration to the spleen and liver has been observed during the extra-intestinal phase (Fernando et al., 1987).

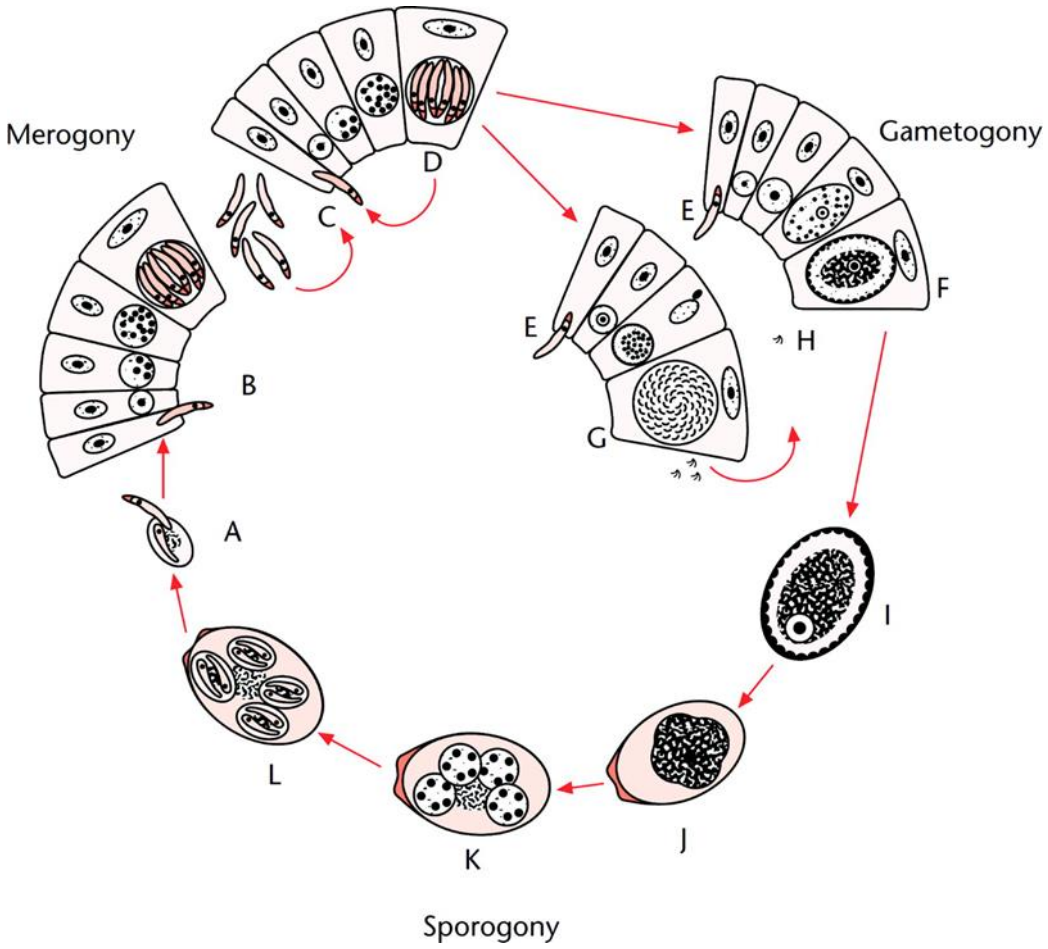

Figure 2. 1. The life cycle of Eimeria. Sporozoites (A); Merogony (B); Merogonic cycles (C – D); Gametogony (E); Macrogamete (F); Microgametocyte (G); Microgamete (H); Unsporulated oocyst (I); Sporogony (I – L); Infective oocyst (L) (Price, 2012).

The time required for excystation of sporozoites to infection of intestinal epithelial cells is around 1-2 h (Parry et al., 1992). The length of time needed for the endogenous development of zygote from sporozoite excystation is 4-7 d (Parry et al., 1992), whereas the exogenous phase takes approximately 2 d to complete the sporogony (Chapman et al., 2002). *Eimeria* species differ in the region of infection (from the duodenum to caecum) and pathogenicity (from mild to severe). *Eimeria* oocysts can be categorized according to the size as small (*E. acervulina*, *E. mivati* and *E. mitis*), medium (*E. necatrix*, *E. tenella* and *E. praecox*) and large (*E. maxima*) (Price, 2012).

2.7.1.2. Pathogenesis and clinical signs

The severity of coccidiosis depends on the number of oocysts that are ingested by the bird. Severe clinical signs are associated with the ingestion of thousands of oocysts, whereas ingestion of hundreds of oocysts leads to subclinical infection (Reid, 1990). Clinical coccidiosis can be associated with the morbidity due to bloody diarrhea, and some mortality. Subclinical coccidiosis causes decreased body weight gain, higher feed conversion ratio, and reduced egg production in laying hens (Vermeulen et al., 2001). The most pathogenic *Eimeria* species from the seven species recognized as infecting broiler chickens are *E. acervulina*, *E. maxima* and *E. tenella*. *E. acervulina* and *E. maxima* are more prevalent in broiler chickens compared to *E. tenella* (De Gusseum, 2007). Coccidiosis disease severity in chickens can vary depending on the species, and magnitude and site of infection (Chapman, 2014). *Eimeria acervulina* causes white pin-point lesions in the duodenum and can extend to more caudal regions at a heavier infection. *Eimeria acervulina* and *E. mitis* cause mild enteritis resulting in fluid loss and malabsorption of nutrients. *E. maxima* causes petechial hemorrhages in the midgut and sloughing of epithelia. *Eimeria tenella* infects the caeca by invading deep mucosa, causing widespread damage and resulting in loss of blood in the faeces and distinct gross lesions in the caeca. *Eimeria necatrix* and *E. tenella* cause complete villi destruction, which results in severe hemorrhage and death. Other *Eimeria* species are difficult to diagnose using macroscopic lesions as they are not associated with distinct gross lesions (De Gusseum, 2007; Chapman, 2014). The infection can cause changes in mucosal permeability resulting in leakage of plasma protein into the intestinal lumen, high pH, and reduced gut motility (Chapman, 2014).

2.7.1.3. Diagnosis

Diagnosis of coccidiosis is difficult and this is the main reason that the disease is still a major problem in the poultry industry (De Gusseum, 2007). Oocyst count, macroscopic lesion and microscopic lesion scoring are the main methods used to diagnose the disease (Goodwin et al., 1998). Oocysts per gram count in faeces or litter is a commonly used technique, but its relationship to bird performance is very low (De Gusseum, 2007). Oocyst count scoring is also performed using the small intestine mucosal scrapings (Goodwin et al., 1998). However, identification of species according to oocyst morphology requires expertise and experience.

Macroscopic lesion scoring is also a common method of diagnosis, but the method is very labour-intensive, subjective and requires expertise. It also has a poor relationship to bird performance especially during a subclinical infection (De Gusseum, 2007). According to Goodwin et al (1998), microscopic lesion scoring is superior to oocyst scoring and macroscopic lesion scoring, as it detects developmental stages of the parasite other than oocysts and helps to recognize other causes for intestinal disease. Biochemical and molecular techniques including polymerase chain reaction (PCR) technique can be used to diagnose coccidiosis and it helps in the diagnosis of the currently ignored species such as *E. praecox* and *E. mitis* (Morris and Gasser, 2006).

2.7.1.4. Prevention and control measures

Good husbandry helps prevention of coccidiosis transmission, but additional strategies are required in controlling the disease. Administration of anticoccidial drugs is a common method used to control coccidiosis in chickens, either using coccidiostatic or coccidiocidal agents. Coccidiostatic drugs prevent the growth and replication of the coccidian species, whereas coccidiocidals destroy the coccidian population. Coccidiocidal drugs are more effective compared to coccidiostatic drugs because parasite multiplication starts with the termination of coccidiostats (Quiroz-Castaneda and Dantan-Gonzales, 2015). There are two categories of drugs use as anti-coccidials namely ionophores (eg: Quinolones) and synthetic drugs (eg: nicarbazin, amprolium). Ionophores interfere with the transportation of ions across the cell membrane of organs in the parasite, arrest the development stages and finally cause death. Synthetic drugs destroy the parasite by inhibiting critical biochemical pathways of the parasite (Chapman et al., 2010). However, the emergence of anti-coccidial drug resistance is a major problem for coccidiosis control especially after the prolonged use of a drug. Shuttle and rotation programs can be used to minimize the resistance of these drugs. Different drugs are used from hatch to market size in a shuttle program, whereas drug type is switched after grow-out periods or seasonally in a rotation program. However, drug resistance cannot be precluded using these programs (De Gusseum, 2007). Anti-coccidial drugs can be withdrawn in a specific period before slaughter of broilers, and also before the commencement of egg production in laying hens

to reduce the potential for drug residues, but then the birds again become susceptible to the infection after ceasing drug usage (Price, 2012).

Live vaccination is a common strategy that used to prevent coccidiosis in chickens. Vaccination causes the induction of a species-specific immune response that needs to protect from a coccidian disease challenge (Price, 2012). There are two types of live vaccines used in the poultry industry, live unattenuated and live attenuated vaccines. Live unattenuated vaccine usage is limited due to the disease-causing risk associated with the live parasite (Williams, 2002). Therefore, live attenuated vaccines are commonly used in the poultry industry as the risk of disease occurrence is very low. Further, proliferation of the parasite is low, which results in less damage to the intestinal mucosa (Sharman et al., 2010). Spray-on-feed and hatchery spray are the common routes of vaccine administration in the poultry industry (Price, 2012). However, the development of an effective anticoccidial vaccine is still a major challenge, because *Eimeria* species distribution varies between poultry farms. Therefore, screenings for *Eimeria* species should be done before administering an anticoccidial vaccine (Quiroz-Castaneda and Dantan-Gonzales, 2015). Vaccines are sometimes used with medication in rotation programs to control *Eimeria* (Peek and Landman, 2011).

Some natural compounds can be used as control measures of coccidiosis in these include fungal extracts, plant extracts and probiotics. They are mainly used as diet supplements. It has been proven that high concentrations of n-3 fatty acids (docosahexaenoic acid, eicosapentaenoic acid and linolenic acid) reduce the severity of *Eimeria* infection in broiler chickens by inducing oxidative stress in the parasite (Allen et al., 1996). In addition, the use of antioxidants from natural sources has been able to improve the condition of birds that are infected with coccidiosis. Some commercial essential oils including carvacrol, carvone, isopulegol, thymol and eugenol were proved as having oocysticidal activity (Remmal et al., 2013). A commercial probiotic which contains *Pediococcus acidilactici* and *Saccharomyces boulardii* has demonstrated as a control method for coccidiosis (Lee et al., 2007). Arabinoxylan, a non-starch polysaccharide derived from wheat also showed immune-stimulatory effect against coccidiosis in broiler chickens (Akhtar et al., 2012).

2.8. Salmonellosis

Salmonellosis is an infectious disease of humans and animals caused by species of *Salmonella* (*S. enterica* and *S. bongori*). It is primarily an intestinal bacterium but can be found in the environment and any material contaminated with faeces. *Salmonella* causes diarrhea and systemic infections in humans because of consuming contaminated foods or exposure in the environment. Subclinical infection of food animals causes contamination of meat, eggs or milk. Human salmonellosis is a very common and economically important zoonotic disease. *Salmonella* infection of food animals is very important in terms of public health and food safety, as the animal originated food is considered as the main source of human salmonellosis. Many animals including poultry get infected with *Salmonella* but show no clinical disease. However, they are involved in spreading the infection through the flock and acting as a source of food contamination (OIE, 2010).

Bacteria of the genus *Salmonella* are gram-negative, facultatively anaerobic, non-spore forming, motile rods, and belong to the Enterobacteriaceae family (Cosby et al., 2015). *Salmonella enterica* is divided into six subspecies (*enterica*, *salamae*, *arizonae*, *diarizonae*, *houtenae* and *indica*) (OIE, 2010). *Salmonella* Enteritidis and *Salmonella* Typhimurium (belong to *S. enterica* subspecies *enterica*) are the most common serotypes that cause foodborne illnesses in humans, and the first and second most common serotypes, respectively (Cosby et al., 2015). *Salmonella enterica* biovars Gallinarum and Pullorum (both belongs to *Salmonella* Gallinarum serovar) are host-specific bacteria that affects poultry and aquatic birds but are not able to cause diseases in mammals (Feng et al., 2013).

2.8.1. Salmonellosis in chickens

The most common sources of Salmonellosis are poultry and poultry by-products including raw and uncooked eggs. Therefore, studying *Salmonella* infection in poultry is very important. *Salmonella* causes asymptomatic infection in chickens. However, some acute infections result in clinical signs and mortality of chicks younger than two weeks old (Suzuki, 1994). Laying hens are predisposed to *Salmonella* and can result in a systemic infection. *Salmonella* Enteritidis have the capability of deposition in the internal contents of eggs by transovarian transmission after colonizing the intestinal tract of chickens (Thiagarajan et al.,

1994). In laying hens, the infection rarely causes mortality in birds aged more than one month (Suzuki, 1994). Most *Salmonella* infections in chicken begin by ingesting the organism. Then the ingested organism passes through the alimentary tract and adheres to and penetrates the intestinal epithelial cells. Sometimes after penetrating intestinal epithelial cells, these organisms invade and multiply in the reticuloendothelial system, and spread into other tissues causing a systemic infection (Barrow et al., 1987).

Contamination of eggs with *Salmonella* Enteritidis can occur by penetration of the organism through the eggshell from contaminated faeces after or during oviposition (Gast and Beard, 1990), or direct contamination of yolk, albumen, shell membranes or eggshell by transovarian infection with the organism before oviposition (Timoney et al., 1989). Faecal shedding of *Salmonella* Enteritidis for an extended time may increase the prevalence of egg soiling with infected droppings (Humphrey et al., 1990). However, research has proven *Salmonella* organisms were isolated more from the yolk and albumen of eggs than from eggshells of infected laying hens (Gast and Beard, 1990).

2.8.2. Pathogenesis

Various virulence factors are associated with *Salmonella* Enteritidis infection in chicken. Enterotoxins of *Salmonella* have been identified as a virulent factor causing infection. Enterotoxins mediate the activation of adenylate cyclase in the cytoplasmic membrane of the host epithelial cells and increase cyclic AMP in the cytoplasm. This leads to the profuse loss of intestinal fluid (Peterson et al., 1983). Cytotoxin production also has been demonstrated in *Salmonella* Enteritidis in chickens. It causes damage to the intestinal mucosa, and results in enteric symptoms including diarrhea (Koo et al., 1984). Lipopolysaccharide of the bacterial cell wall is another virulence factor, and activates macrophages resulting in phagocytosis and inflammatory signs. The organism's adherence to host cells, invasion, intracellular survival, and multiplication also are involved in the pathogenesis of *Salmonella* infection in chicken (Suzuki, 1994).

2.8.3. Pathology

Chickens infected with *Salmonella* Enteritidis show pericarditis, necrotic foci in the liver and an indurated yolk sac remnant in young chicks (Barrow, 1991). Infected hens have deformed, shrunken, discoloured and/or congested ovaries and ovules, and shrunken, malformed follicles with fluid-filled cysts attached, soft-shelled eggs and egg peritonitis (Hopper and Mawer, 1988). These pathological lesions confirm the transovarian transmission of *Salmonella* Enteritidis infection in chicken. Peritonitis, pericarditis, small necrotic foci in the liver and kidneys suggest the systemic infection of Salmonellosis in chickens (Suzuki, 1994).

2.8.4. Diagnosis

Salmonella organisms can be isolated by culture methods using pre-enrichment media, enrichment media and selective plating media. The presence of *Salmonella* in faecal samples of asymptomatic animals, and environment or food samples is at a low level, and the organism can be killed by the toxic effects of selective culture media. Thus, pre-enrichment media (e.g. - peptone buffered water) cause multiplication of bacteria or help to resuscitate the organisms that have been damaged. Enrichment media are liquid or semi-solid agar media (e.g. - brilliant green broth, Rappaport-Vassiliadis broth) that contain additives that selectively let *Salmonella* grow, while inhibiting the growth of other bacteria. Selective plating media are solid agar (eg- brilliant green agar, deoxycholate/citrate agar, MacConkey agar) that permit differential growth and give information on some biochemical characteristics. Serological tests including whole blood test, rapid slide agglutination test, serum agglutination test and ELISA also can be used to diagnose *Salmonella* infection (OIE, 2010).

2.8.5. Prevention and control measures

Vaccination is the most common method used to control Salmonellosis with the reduction of in-feed antibiotic usage. The goal of vaccinating laying hens is to reduce/suppress egg contamination. Live attenuated vaccines and bacterins are the two types of *Salmonella* vaccines that are used in the poultry industry. They can be administered through the drinking water (De Buck et al., 2004). Vaccination is usually administered to broiler parents to prevent susceptibility of offspring (Eeckhaut et al., 2008). The use of prebiotics, probiotics and synbiotics are the other

control measures that can be implemented to help prevent infection (Van Immerseel et al., 2002). Eeckhaut et al. (2008) observed a reduction of *Salmonella* Enteritidis colonization in the caeca, and also a reduction of *Salmonella* translocation to the spleen in broiler chickens with the dietary addition of AXOS. Research has proven AXOS increases the caecal bifidobacteria count (Courtin et al., 2008b). In turn bifidobacteria have the potential of increasing butyric acid production which stimulates the growth of strictly anaerobic bacteria (Belenguer et al., 2006). Further, it decreases the invasion of *Salmonella* in gut epithelial cells and colonization of the caeca and internal organs (Van Immerseel et al., 2004b). High levels of mixed linked β-glucan in HB reduced the abundance of *Salmonella* Typhimurium in the ileum, caeca and colon of pigs according to Pieper et al. (2012). Fermentable carbohydrates including β-glucan are used by the favourable bacteria in the gut and may lead to the reduction of substrate for pathogenic bacteria. Therefore, numbers of disease-causing bacteria including *Salmonella* colonization are reduced. In addition, HB β-glucan increased the concentration of propionate, and propionate has antimicrobial properties against pathogenic bacteria including *Salmonella*. Tellez et al. (1993) observed a reduction of *Salmonella* Enteritidis organ colonization in chickens with the supplementation of dietary lactose. Dietary lactose is a disaccharide that cannot get digested in chickens, and therefore it reaches the hind gut and undergoes bacterial fermentation. It causes enhancement of SCFA production and an improvement in the caecal morphology which ultimately decreases *Salmonella* colonization. Fructo-oligosaccharides also have the potential for the reduction of *Salmonella* Typhimurium colonization in the chicken intestine (Bailey et al., 1991). *Salmonella* Typhimurium colonization in the caeca was reduced in broiler chickens with dietary supplementation of MOS compared to the control diet (Spring et al., 2000).

2.9. Summary

The effect of exogenous BGase on digesta viscosity in chickens, and the subsequent effect on nutrient digestibility and production performance is very well-studied in the literature (Karunaratne and Classen, 2019). Therefore, dietary addition of BGase is a common practice in the poultry feed industry to minimize the negative effects associated with cereal β-glucan when the birds are fed barley- or oat-based diets. Beta-glucanase mediated reduction of digesta viscosity might be associated with β-glucan depolymerization. However, the molecular weight of

β-glucan in the digestive tract content has not been assessed in the previous poultry research to establish the mechanism of the enzyme. Therefore, investigation of soluble β-glucan molecular weight in the chickens is a major research focus in the current study. Beta-glucanase dose response has not been studied in chickens in the literature but is important to determine since increasing dose might further affect β-glucan depolymerization and resulting in a higher proportion of low molecular weight carbohydrates in the lower digestive tract especially in the ileum.

Xylanase effect on digesta viscosity, and on bacterial fermentation and other digestive physiological parameters is well studied in the previous research (Bedford, 2018), since the predominance of arabinoxylan in the most important cereal grains in poultry diets (wheat and corn). However, β-glucan has received less attention due to the the limited use of barley and oats in poultry feeding. Endoxylanase depolymerizes arabinoxylan into xylo- and arabinoxylo-oligosaccharides, and increase bacterial fermentation, and thereby exerts prebiotic properties in chickens (Bautil et al., 2019). Therefore, it is logical to investigate the effect of a high dose of feed BGase on carbohydrate fermentation through the reduction of soluble digesta β-glucan molecular weight in the current research, in addition to the BGase effect on digesta viscosity that has already been well-established (Salih et al., 1991; Fuente et al., 1995; Józefiak et al., 2005; 2006).

There is very minimal research that differentiates the effect of xylanase and BGase in chickens, and it was only tested in few viscosity and production trials (Dos Santos et al., 2013; Mathlouthi et al., 2002b). Therefore, this is the first time in poultry research that use of a relatively purified form of feed BGase is being explored to test its' dose effect on β-glucan depolymerization, and digestive tract physiology and morphology in chickens. Further, the minimal research in the literature regarding BGase's effect on bacterial fermentation shows less and inconsistent effects across the studies in chickens (Józefiak et al., 2005; 2006), and warrants further investigation. Moreover, BGase effects on bacterial fermentation is an important assessment to determine its' involvement on other digestive tract characteristics (gut morphology, digestive tract gene expression) and production.

Finding alternatives to antibiotics is a current research focus in the poultry industry (Ducatelle et al., 2015; Gadde et al., 2017), and therefore the current study also determines the ability of BGase to replace diet medication when the birds were fed HB-based diets. Enteric diseases in poultry is a major issue with the reduction of in-feed antibiotics usage (Diarra and Malouin, 2014), and therefore the current research also investigate HB and BGase effects under disease-challenge conditions in addition to the experiments on un-challenged birds.

The overall objective of the research was to investigate the effects of HB and exogenous BGase on soluble digesta β-glucan molecular weight distribution, digestive tract morphology and physiology, and production performance in chickens. Further, the investigation of diet medication and BGase on the same aforementioned parameters was another objective of the study to address the concerns regarding antibiotic-free poultry production, and to test the ability of BGase to replace diet medication. In addition, the effects of dietary BGase and HB on *Salmonella* Enteritidis colonization and translocation in broiler chickens will also come under the overall objectives of the study.

3.0 EFFECTS OF DIET HULLESS BARLEY AND BETA-GLUCANASE LEVELS ON ILEAL DIGESTA SOLUBLE BETA-GLUCAN MOLECULAR WEIGHT, DIGESTIVE TRACT CHARACTERISTICS AND PERFORMANCE OF BROILER CHICKENS

3.1 Abstract

The reduction of antibiotics use in poultry feed has led to the investigation of alternatives to antibiotics, and one such substitution is fermentable carbohydrates. Exogenous β-glucanase (BGase) use is a common practice in poultry fed barley-based diets to reduce digesta viscosity. The objective of the study was to determine the effects of diet hulless barley (HB) and BGase levels on ileal digesta soluble β-glucan molecular weight (MW), digestive tract characteristics and performance of broiler chickens. A total of 360, day-old broilers were housed in battery cages, and fed graded levels of high β-glucan HB (CDC Fibar; 0, 30 and 60% replacing wheat) and BGase (Econase GT 200 P from ABVista, Wiltshire, UK; 0, 0.01 and 0.1%) in a 3 × 3 factorial arrangement. Each treatment was assigned to 10 cages with 4 birds in each cage. Performance was assessed weekly, and the sample collection was completed by d 28. Peak MW of β-glucan in the ileal digesta was lower with 30 and 60 compared to 0% HB (0% HB;18045^{a}, 30% HB;13953^{b}, 60% HB;14162^{b}), whereas decreased with increasing BGase. Weight average MW was lower at 0.1 compared to 0 and 0.01% BGase in wheat diets, whereas in 60% HB diets, it was lower with 0.01 and 0.1 compared to the 0% BGase. The maximum MW was lower with 0.01 and 0.1 than the 0% BGase despite the level of HB. Further, maximum MW was lower with HB compared to wheat when the diets contain 0 or 0.01% BGase. Overall, empty weights and lengths of GIT sections increased with increasing HB, but there was no BGase effect. Hulless barley decreased the duodenum and jejunum contents, whereas increased the contents of gizzard (diets with BGase), ileum and colon. Jejunum and small intestine content decreased with increasing BGase. Pancreas weight increased with increasing HB, and 0.1% BGase decreased the

weight compared to 0.01% BGase. Ileal and colon pH increased with increasing HB, but there was no BGase effect. Treatment effects were minor on SCFA levels and performance. In conclusion, exogenous BGase depolymerized high MW ileal digesta β-glucan of broilers. However, HB and BGase did not affect carbohydrate fermentation in the ileum and caeca, although there were some significant treatment effects on the digestive tract characteristics and performance of broilers.

Keywords: prebiotics, viscosity, oligosaccharides, non-starch polysaccharidases, fibre

3.2 Introduction

The emergence of antibiotic-resistant pathogenic organisms due to the continued use of antibiotics in feed is a major concern in the poultry industry (Diarra et al., 2007; Furtula et al., 2010; Kaesbohrer et al., 2012; Garcia-Migura et al., 2014; Roth et al., 2019). Therefore, usage of in-feed antibiotics is getting reduced, and the identification of alternative strategies to the usage of antibiotics has become a major research focus. Especially the effect of these alternatives to antibiotics on gastro-intestinal (GI) health of chickens including microbial composition, intestinal morphology and immune response, and production performance has been investigated in many studies (Gadde et al., 2017; Mehdi et al., 2018; Suresh et al., 2018). Prebiotics is one of the most common alternatives to antibiotics that has been extensively studied in poultry (Ducatelle et al., 2015; Pourabedin and Zhao, 2015; Adhikari and Kim, 2017).

Prebiotics are non-digestible nutrient compounds that undergo microbial metabolization in the gastro-intestinal tract (GIT), and results in beneficial physiological effects on the host via different mechanisms (Bindels et al., 2015). However, the most recent definition for prebiotics is "a substrate that is selectively utilized by host microorganisms conferring a health benefit" according to the International Scientific Association of Probiotics and Prebiotics (Gibson et al., 2017). The mechanisms of prebiotics that contributes to the host health includes change of gut microbial population through competitive exclusion (Ofek and Beachey, 1978; Baurhoo et al., 2007; Rebole et al., 2010; Kim et al., 2011), modification of GI morphology (Baurhoo et al., 2009; Chee et al., 2010; Pourabedin et al., 2014; Shang et al., 2015) and immune function (Yitbarek et al., 2012; Huang et al., 2015) through increased carbohydrate fermentation

(Keerquin et al., 2017; Józefiak et al., 2005). The most common type of prebiotics that has been studied in literature is fermentable carbohydrates, and they are namely fructo-oligosaccharides (FOS), inulin type fructans, mannan oligosaccharides (MOS) and arabinoxylo/xylo-oligosaccharides (AXOS/XOS) (Pourabedin and Zhao, 2015). Dietary addition of wheat AXOS and XOS have been extensively studied in literature, and observed modification of gut microbiota, carbohydrate fermentation, immune function and improved performance of chickens (Courtin et al., 2008 a; b; Eechhaut et al., 2008; De Maesschalck et al., 2015; Keerquin et al., 2017; Ding et al., 2018).

The current study focused on the prebiotic effect of low MW β-glucan of hulless barley (HB) in chickens. Previous research demonstrates increased production performance of chickens fed barley-based diets with β-glucanase (BGase) supplementation (Edney et al., 1989; Classen et al., 1988a; Campbell et al., 1989) due to the reduction of digesta viscosity (Salih et al., 1991; Fuente et al., 1995; Mathlouthi et al., 2002a), and thereby increasing nutrient digestibility (Hesselman and Åman, 1986; Edney et al., 1989; ; Perttila et al., 2001) and modifying microbial population in the GIT (Choct et al., 1999; Józefiak et al., 2006; 2010). Barley-based diets with the supplementation of BGase affected carbohydrate fermentation in the ileum and caeca, and modulated GI microbial ecology in broiler chickens (Józefiak et al., 2005; 2006; 2010), which supports the beneficial effect of BGase supplemented barley β-glucan on microbial fermentation. However, the mechanism of BGase on barley β-glucan that affect the carbohydrate fermentation is less well studied in the literature. Further, BGase that was used in the previous research regarding carbohydrate fermentation was not purified and consisted of similarly high level of endoxylanase (Józefiak et al., 2005; 2006), which is not demonstrating the individual and exact effect of BGase due to the release of both β-glucan and arabinoxylan. In addition, prebiotic effect of most of the fermentable carbohydrates including fructose, mannose and arabinoxylan have been tested with the extracted and low molecular weight (MW) form (Kim et al., 2011; Pourabedin and Zhao, 2015). Therefore, the low MW β-glucan of HB might be a beneficial fermentable carbohydrate in terms of improving GI physiology especially carbohydrate fermentation, GI microbial population and production performance of chickens. Moreover, the

use of a purified form of exogenous BGase helps to understand the pure effect of BGase on soluble β-glucan MW and digestive tract characteristics of broiler chickens.

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

3.3 Materials and methods

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

3.3.1 Birds and housing

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

3.3.2 Experimental diets

The dietary treatments were designed according to a 3 × 3 factorial arrangement. Hulless barley (CDC Fibar; β-glucan – 8.7%) level and BGase (Econase GT 200 P from ABVista, Wiltshire, UK) level were the two factors with HB levels of 0, 30 and 60%, and BGase levels of 0 (0 BU/kg), 0.01 (20,000 BU/kg) and 0.1 (200,000 BU/kg) % were included in the diets. Hulless barley was added by replacing wheat in each diet, assuming both ingredients have an approximately similar nutrient composition. Diets were formulated to meet or exceed Ross 308 broiler nutrition specifications (Aviagen 2014) and were fed in crumble form throughout the trial. The ingredients and calculated nutrient levels are presented in Table 3.1. Titanium oxide was used as an indigestible marker to determine AME and β-glucan digestibility. The pelleting temperature was maintained at 70-75°C to prevent BGase inactivation due to high temperature during feed processing. Beta-glucanase (EC 3.2.1.6) and xylanase activity (EC 3.2.1.8) of the diets were analyzed according to the AB Vista methods of ESC Standard Analytical Method SAM042-01 and SAM038 respectively. The analyzed enzyme activity approximated the calculated values based on the enzyme addition, which confirms the correct addition of BGase to the diets, and the enzyme activity was not lost following feed processing. Further, xylanase activity was non-detectable.

Table 3. 1. Ingredients and calculated nutrient levels of experimental diets

Ingredient	Quantity (%)
Cereal grain (Wheat or hulless barley)[1]	60.00
Wheat (remaining)	5.00
Soybean meal	26.93
Canola oil	4.07
Mono-dicalcium phosphate	1.20
Limestone	1.52
Sodium chloride	0.38
Vitamin-mineral premix[2]	0.50
Choline chloride	0.10
TiO_2	0.30
Nutrient, calculated	
AME (kcal/kg)	3100
Crude protein	21.24
Crude fat	5.57
Calcium	0.87
Chloride	0.36
Non-phytate phosphorous	0.44
Potassium	0.83
Sodium	0.18
Digestible arginine	1.35
Digestible Isoleucine	0.81
Digestible leucine	1.47
Digestible lysine	1.15
Digestible methionine	0.54
Digestible methionine and cysteine	0.87
Digestible threonine	0.77
Digestible tryptophan	0.24
Digestible valine	0.87

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

[2]Vitamin-mineral premix provided the following per kilogram of 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 3773 mg.

3.3.3 Performance data collection

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

3.3.4 Sample collection

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

All the birds were euthanized at d 28 by intravenous administration of T-61 consisted of Embutramide, mebezonium iodide and tetracaine hydrochloride (Merck animal health, Kirkland, Quebec, Canada) into the brachial vein, and the birds were weighed individually. Two birds per cage were used to collect samples for the analysis of SCFA. Entire ileal and caecal contents were collected into plastic centrifuge tubes and stored in -20°C for the analysis of SCFA. The pH of crop, gizzard, duodenum, jejunum, ileum, caeca, and colon contents was measured *in situ* using a Beckman Coulter 34 pH meter (Model PHI 34, Beckman Instruments, Fullerton, CA) before collecting contents for the SCFA analysis. Two birds per cage were used to obtain digestive tract size and organ weights. The digestive tract was removed from the bird carcass, and then separated into the crop, proventriculus, gizzard, duodenum, jejunum, ileum, caeca, and colon; organs (liver, spleen, and pancreas) were removed at the same time. Full weight, empty weight, and the length (when appropriate) of each section of the digestive tract, and organ weights were recorded. The content weight of each section was determined by subtracting the empty weight from the full weight. Empty weight, length, content, and organ weight were divided by individual bird weight to obtain the relative parameters. Ileal contents were collected into plastic snap-cap vials (pooled the contents from two birds per cage), and a portion of it was centrifuged for 3-5 min at 14,000 rpm using a Beckman microfuge (Model E348720, Beckmann instruments, INC, Palo Alto, CA). The viscosity of the pooled ileal supernatant was measured using a Brookfield digital viscometer (Model LVDV-III, Brookfield Engineering Labs, INC, Stoughton,

MA 02072). The rest of the ileal supernatant derived from centrifugation was stored at -80°C for the analysis of β-glucan MW distribution.

3.3.5 Nutritional analysis of experimental diets, excreta and ileal digesta

Experimental diets, ingredients (HB and wheat) and excreta were ground to 1 mm (for GE, N, fat, ash, minerals, Ti, and insoluble and soluble dietary fibre analyses) and 0.5 mm (for β-glucan and total starch analyses) screen-hole sizes using a Retsch laboratory mill (Retsch ZM 200, Germany). Ileal digesta was ground using mortar and pestle after freeze-drying. Diets, excreta and ileal digesta were analyzed for β-glucan, Ti and moisture. Diets and excreta were analyzed for gross energy (GE) and nitrogen (N). Beta-glucan was analyzed (AOAC Method 995.16, AACC Method 32-23 and ICC Standard Method No. 168) using a Megazyme analysis kit (Mixed-linkage beta-glucan assay procedure/McCleary method, Megazyme International Ireland Ltd., Bray Business Park, Bray, Co. Wicklow, Ireland). Titanium was determined according to the procedure described by Myers, (2014) and moisture was analyzed using AOAC method 930.15. Gross energy was determined using an oxygen bomb calorimeter (Model A1435DDEB, Parr Instruments, Moline, IL, USA). Nitrogen was analyzed using a Leco nitrogen analyzer (Model Leco-FP-528L, Leco Corporation, St. Joseph, MA, USA), and 6.25 was used as the N to CP correction factor. In addition, diets were analyzed for total starch, fat, ash, insoluble dietary fibre (IDF) and soluble dietary fibre (SDF). The ingredients were analyzed for IDF, SDF, β-glucan, total starch, CP, fat, minerals, and moisture. Insoluble dietary fibre and SDF were analyzed using a Megazyme kit (Total dietary fibre assay procedure, Megazyme International Ireland Ltd., Bray Business Park, Bray, Co. Wicklow, Ireland) according to the AOAC method 991.43 and AACC method 32-07.01. Total dietary fibre (TDF) was calculated by adding IDF and SDF. Fat content was determined by ethyl ether extraction using Goldfish Extraction Apparatus (Labconco model 35001; Labconco, Kansas, MO, USA) following the AOAC method 920.39. Ash content was analyzed according to AOAC method 942.05 using a muffle oven (Model Lindberg/Blue BF51842C, Asheville, NC 28804, USA). Experimental diets were analyzed for the enzyme activity (both BGase and xylanase) according to ESC Standard Analytical Method SAM042-01 and SAM038 respectively by ABVista (ABVista, Wiltshire, UK).

3.3.6 Beta-glucan molecular weight distribution

Ileal supernatant was analyzed for β-glucan MW using size exclusion chromatography followed by calcofluor post-column detection for fluorescent recognition (Boyd et al., 2017). The two columns used for HPLC were the Shodex OHpak SB-806M column with OHpak SB-G guard column and a Waters Ultrahydrogel linear column. Tris buffer (0.1M; pH=8) was used as the mobile phase. Beta-glucan peak molecular weight (Mp) and weight average molecular weight (Mw) of each sample were determined using a molar mass distribution curve. The molecular weight of the most abundant β-glucan proportion is referred as Mp and the average (based on the weight fraction of each type of molecules) of the molecular weights of all the β-glucan molecules is the definition for Mw. In addition, the maximum molecular weight of the smallest 10% β-glucan molecules (MW-10%) was detected using the same distribution curve. Ileal supernatant was boiled for 15 min to inactivate endogenous BGase activity in the sample, and then centrifuged for 5 min at 10,000 rpm using a Beckman microfuge (Model E348720, Beckmann instruments, INC, Palo Alto, CA) before loading the samples into HPLC.

3.3.7 Short chain fatty acids analysis

Short chain fatty acids were analyzed according to a slightly modified method of Zhao et al. (2006). The internal standard was made using 20 ml of 25% phosphoric acid, 300 µl of isocaproic acid and deionized water. The standard solution consisted of 300 µl of acetic acid, 200 µl of propionic acid, 100 µl of butyric acid and 50 µl of isobutyric, isovaleric, valeric, caproic and lactic acids. Digesta samples were thawed and phosphoric acid was added at a ratio of 1:1 into it. It was mixed and centrifuged at 12,000 rpm for 10 min. Then three aliquots of 1 ml supernatant were taken and mixed with 1 ml of the internal standard. They were filled into microcentrifuge tubes and centrifuged at 16,000 rpm for 10 min. The supernatant was filtered using a syringe and a 0.45 µm nylon filter, and then injected into the Gas Chromatography column. Thermo Scientific Gas Chromatograph (Model Trace 1310, Milan, Italy) with Zebron Capillary Gas Chromatography column (ZB-FFAP, length: 30m; internal diameter: 0.25 mm; film thickness: 0.25 µm, Phenomenex, Torrance, CA) was used for the analysis.

3.3.8 Beta-glucan digestibility and AME_n calculation

Beta-glucan digestibility in the ileum and excreta was calculated using the following equation that was developed according to Weurding et al. (2001) starch digestibility equations.

Beta-glucan digestibility (%) = $1 - [(\%\ Ti_{diet} \div \%\ Ti_{ileum}) \times (\%\ \beta\text{-glucan}_{ileum} \div \%\ \beta\text{-glucan}_{diet})] \times 100$……...(3.1)

The total tract β-glucan digestibility was calculated by replacing $\beta\text{-glucan}_{ileum}$ with $\beta\text{-glucan}_{excreta}$ of the above equation.

Nitrogen corrected AME was determined using Hill and Anderson, (1958) equations.

$AME_{n\ (Cal/g.diet)} = AME_{Cal/g.diet} - (8220 \times ANR_{g/g.diet})$……...(3.2)

$AME_{Cal/g.diet} = GE_{Cal/g.diet} - [GE_{Cal/g.excreta} \times (\%\ Ti_{diet} \div \%\ Ti_{excreta})]$……..……(3.3)

$ANR_{g/g.diet} = N_{g/g.diet} - [N_{g/g.excreta} \times (\%\ Ti_{diet} \div \%\ Ti_{excreta})]$……..…….(3.4)

Where:

$ANR_{g/g.diet}$ = Apparent N Retained (g/g of diet)

8220 = Correction factor (Cal per g N retained in the body)

3.3.9 Statistical analysis

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

3.4 Results

3.4.1 Ingredient nutrient composition

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

3.4.2 Beta-glucan molecular weight distribution

An interaction between HB and BGase was found for the β-glucan Mw and MW-10% of the soluble ileal digesta of broiler chickens (Table 3.2). In the birds fed 0% HB diets, Mw was lower with the addition of 0.1% BGase compared to the 0% BGase. In addition, MW-10% decreased with increasing BGase in the diets. For the 30% HB treatments, Mw decreased with the increasing level of BGase, whereas MW-10% was lower with 0.01 and 0.1% BGase compared to 0% BGase. When considering the 60% HB, both Mw and MW-10% were lower with 0.01 and 0.1% BGase levels than with the 0% BGase. However, no interaction was noticed for Mp of soluble ileal β-glucan. Thus, Mp was higher with 0 compared to 30 and 60% HB levels. Moreover, Mp decreased with the increasing level of BGase in the diets. Figures 3.1A and 3.1B indicates ileal soluble β-glucan MW distribution when the broilers were fed 60% HB without and with BGase, respectively. The proportions of the left side of the blue lines at the point $1e^4$ in each curve demonstrates the proportion of low MW β-glucan has been increased with the addition of 0.1% BGase to the diets, which is in accordance to the MW-10% results in the current study.

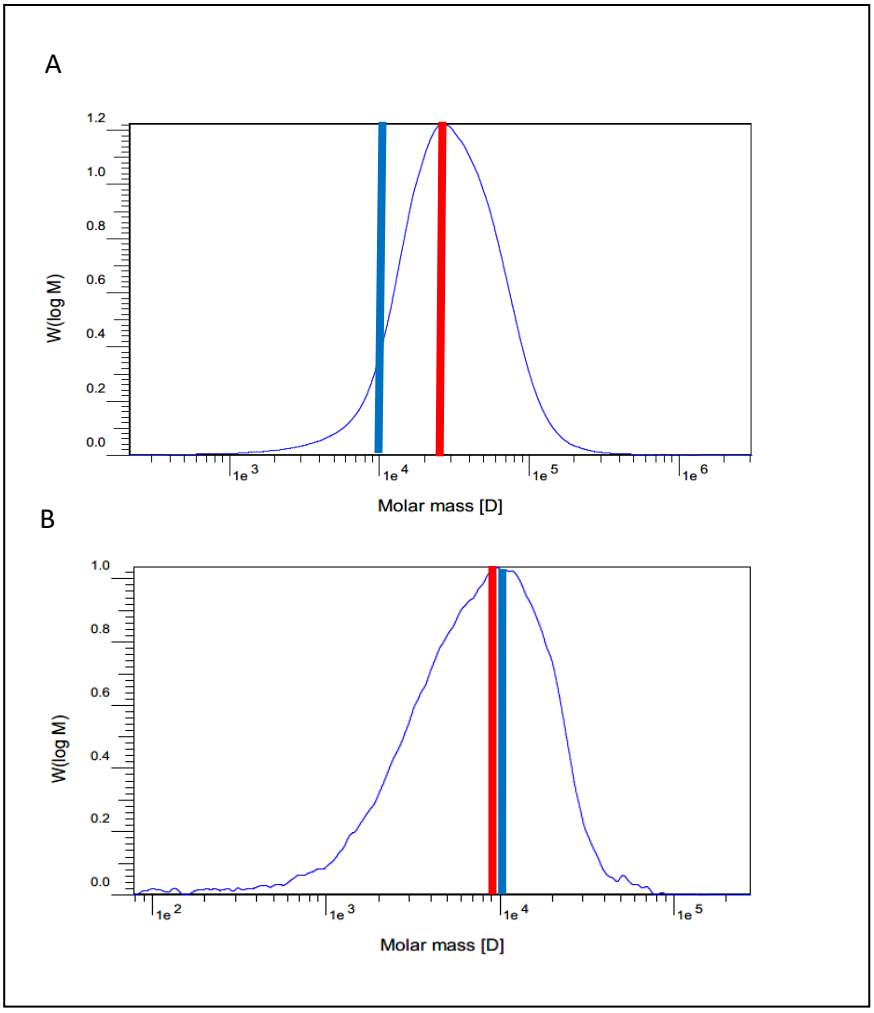

Figure 3. 1. Beta-glucan molecular weight distribution in soluble ileal digesta from broilers fed 60% hulless barley diets. Blue lines denote point 1e4 on the x-axis and red lines indicate the Mp of the distribution curve. A. 0% β-glucanase; B. 0.1% β-glucanase

Table 3. 2. Effects of hulless barley and β-glucanase on β-glucan molecular weight in the ileal content of broiler chickens aged 28 d

Hulless barley (%)	β-glucanase (%)	Molecular weight (g/mol)		
		Mp[1]	Mw	MW-10%
0	0	21536	30486[ab]	9414[a]
	0.01	18276	22427[bc]	5702[b]
	0.1	14324	14620[cd]	2342[c]
30	0	19652	35863[a]	5584[b]
	0.01	13255	22217[bc]	3359[c]
	0.1	8952	10347[d]	2025[c]
60	0	19799	36199[a]	6099[b]
	0.01	14893	16948[cd]	3407[c]
	0.1	7793	8434[d]	1955[c]
SEM[2]		746.8	1507.8	363.7
Main effects				
Hulless barley (%)				
0		18045[a]	22511	5819
30		13953[b]	22809	3656
60		14162[b]	20527	3820
β-glucanase (%)				
0		20329[a]	34183	7032
0.01		15475[b]	20531	4156
0.1		10356[c]	11134	2107
Probability				
Hulless barley		<.0001	0.3628	<.0001
β-glucanase		<.0001	<.0001	<.0001
Hulless barley × β-glucanase		0.2055	0.0349	0.0007

[a-d]Means within a main effect or interaction not sharing a common superscript are significantly different ($P \leq 0.05$).
[1]Mp - peak MW; Mw - weighted average MW; MW-10% - maximum MW for the smallest 10% molecules.
[2]SEM - pooled standard error of mean (n=6 cages per treatment).

3.4.3 Beta-glucan digestibility and AME$_n$

Hulless barley and BGase interaction was significant on β-glucan digestibility of the ileum and total tract (Table 3.3). In the ileum, β-glucan digestibility was higher with 0.1 compared to 0 and 0.01% BGase when the birds were given a wheat-based diet. There was no

significant effect of BGase on β-glucan digestibility at 30% HB level. However, at 60% HB level, β-glucan digestibility was lower with 0.01 compared to 0 and 0.1% BGase levels. In addition, the digestibility was higher in 30 and 60 compared to 0% HB level when the diets did not contain BGase. Total tract β-glucan digestibility was affected by HB and BGase in an exactly similar pattern to the ileal β-glucan digestibility. In addition, the interaction of HB and BGase was significant on AME_n of the broiler chickens. Nitrogen corrected AME decreased with the increasing level of HB when the birds were fed diets without BGase. There was no effect of BGase on AME_n of the broiler chickens at 0 and 30% HB levels. However, AME_n was higher with 0.1 compared to 0 and 0.01% BGase of the broiler chickens fed 60% HB.

Table 3. 3. Effects of hulless barley and β-glucanase on β-glucan digestibility and nitrogen corrected apparent metabolizable energy of broiler chickens aged 28 days

Hulless barley (%)	β-glucanase (%)	β-glucan digestibility (%)		AME_n (90% DM basis)
		Ileum	Total tract	
0	0	25.9[c]	32.4[cd]	3001[a]
	0.01	24.0[c]	32.5[cd]	2938[ab]
	0.1	49.8[a]	69.1[a]	2964[ab]
30	0	42.2[ab]	45.0[b]	2898[b]
	0.01	30.7[bc]	35.4[bc]	2938[ab]
	0.1	42.4[ab]	37.4[bc]	2954[ab]
60	0	51.4[a]	36.9[bc]	2724[c]
	0.01	30.1[bc]	23.4[d]	2704[c]
	0.1	47.5[a]	42.5[bc]	2888[b]
SEM[1]		1.50	1.54	12.4
Main effects				
Hulless barley (%)				
0		33.2	44.7	2967
30		38.4	39.2	2930
60		43.0	34.2	2772
β-glucanase (%)				
0		39.8	38.1	2874
0.01		28.3	30.4	2860
0.1		46.5	49.6	2935
Probability				
Hulless barley		0.0005	<.0001	<.0001
β-glucanase		<.0001	<.0001	<.0001
Hulless barley × β-glucanase		<.0001	<.0001	<.0001

[a-d]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 (n=10 cages per treatment).

3.4.4 Viscosity

Hulless barley and BGase interaction was significant on the viscosity of the ileal content (Table 3.4). The ileal viscosity was lower with 0.1 compared to 0 and 0.01% BGase at both 30 and 60% HB levels. Further, 0.01 and 0.1% BGase resulted in lower ileal viscosity compared to the 0% BGase when the birds were given a wheat-based diet. However, HB effect was not significant on the ileal viscosity apart from higher viscosity at 30 compared to 0 and 60% HB at 0.01% BGase.

Table 3. 4. Effects of hulless barley and β-glucanase on ileal viscosity of broiler chickens aged 28 days

Hulless barley (%)	β-glucanase (%)	Viscosity (cP)
	0	5.39[a]
0	0.01	4.19[bcd]
	0.1	3.78[cd]
	0	5.00[ab]
30	0.01	5.31[a]
	0.1	3.53[d]
	0	4.75[abc]
60	0.01	3.90[cd]
	0.1	3.33[d]
SEM[1]		0.114
Main effects		
Hulless barley (%)		
0		4.45
30		4.61
60		3.99
β-glucanase (%)		
0		5.04
0.01		4.47
0.1		3.54
Probability		
Hulless barley		0.006
β-glucanase		<.0001
Hulless barley × β-glucanase		0.01

[a-d]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 (n=10 cages per treatment).

3.4.5 Short chain fatty acids and gastro-intestinal pH

There were no treatment effects on the ileal and caecal SCFA of broiler chickens except the ileal isobutyric acid (concentration and molar percentage), which had an interaction, but with less clear trends (Table 3.5 and 3.6). In addition, the ileal valeric acid percentage increased with increasing BGase. The concentration of valeric acid also tended to decrease (P = 0.07) with increasing BGase in the diets. A trend was observed for the interaction of main effects on the caecal acetic acid percentage (P = 0.07) but had minor differences. Ileal and colon pH were higher with 30 and 60 compared to 0% HB. Further, a trend was noticed for the interaction on the gizzard and caecal pH (P = 0.06-0.7), but with less clear differences (Table 3.7).

Table 3. 5. Effects of hulless barley and β-glucanase on ileal short chain fatty acids of broiler chickens aged 28 days

HB[1] (%)	BGase (%)	SCFA µmol/g of wet ileal content									Molar percentage of total SCFA							
		Total	Ace	Pro	Buty	Isob	Val	Isov	Cap	Lac	Ace	Pro	Buty	Isob	Val	Isov	Cap	Lac
	0	175.3	65.2	24.4	11.2	2.3^{ab}	3.4	3.1	1.5	63.8	37.2	13.9	6.4	1.3^{ab}	1.9	1.7	0.8	36.3
0	0.01	172.9	64.9	24.1	11.2	2.2^{ab}	2.9	3.0	1.4	62.9	37.5	13.9	6.4	1.2^{ab}	1.7	1.7	0.8	36.3
	0.1	164.5	62.1	23.2	10.3	1.8^{ab}	2.2	2.4	1.0	61.0	37.9	14.1	6.3	1.0^{ab}	1.3	1.4	0.6	37.0
	0	160.3	61.9	22.1	10.0	1.9^{ab}	2.6	2.5	1.0	58.0	38.7	13.4	6.4	1.2^{ab}	1.6	1.5	0.6	36.2
30	0.01	167.5	63.2	22.6	10.7	2.8^{ab}	2.6	2.5	1.1	61.8	37.9	13.3	6.3	1.5^{ab}	1.5	1.5	0.6	37.0
	0.1	171.1	65.4	23.3	10.9	1.6^{ab}	2.4	2.7	1.2	63.2	38.2	13.4	6.4	0.9^{ab}	1.4	1.5	0.7	37.2
	0	165.9	61.8	22.3	10.5	2.7^{ab}	3.3	2.9	1.3	60.6	37.5	13.1	6.4	1.6^{ab}	1.9	1.7	0.7	36.6
60	0.01	182.5	70.4	25.8	11.3	1.1^{b}	2.8	2.8	1.3	66.6	38.5	14.1	6.2	0.7^{b}	1.5	1.5	0.7	36.4
	0.1	157.2	59.1	20.8	10.3	2.9^{a}	2.6	2.2	1.0	58.0	37.6	13.3	6.5	1.8^{a}	1.6	1.4	0.6	36.9
SEM[2]		3.15	1.17	0.51	0.24	0.13	0.12	0.12	0.05	1.16	0.14	0.16	0.08	0.07	0.06	0.06	0.02	0.15
Main effects																		
HB (%)																		
0		170.9	64.1	23.9	10.9	2.1	2.9	2.8	1.3	62.6	37.5	14.0	6.4	1.2	1.6	1.6	0.7	36.5
30		166.3	63.5	22.7	10.5	2.1	2.5	2.6	1.1	61.0	38.3	13.3	6.4	1.2	1.5	1.5	0.6	36.8
60		168.5	63.8	23.0	10.7	2.3	2.9	2.7	1.2	61.7	37.9	13.5	6.4	1.4	1.7	1.5	0.7	36.6
BGase (%)																		
0		167.2	63.0	22.9	10.6	2.3	3.1	2.8	1.3	60.8	37.8	13.5	6.4	1.4	1.8^{a}	1.7	0.7	36.4
0.01		174.3	66.2	24.2	11.0	2.0	2.8	2.8	1.3	63.7	38.0	13.8	6.3	1.1	1.6^{ab}	1.6	0.7	36.6
0.1		164.2	62.2	22.5	10.5	2.1	2.4	2.4	1.1	60.7	37.9	13.6	6.4	1.2	1.4^{b}	1.4	0.6	37.0
Probability																		
HB		0.82	0.98	0.55	0.78	0.85	0.40	0.63	0.43	0.83	0.14	0.24	0.97	0.54	0.47	0.81	0.51	0.79
BGase		0.37	0.31	0.34	0.60	0.60	0.07	0.34	0.17	0.44	0.86	0.79	0.92	0.40	0.05	0.35	0.13	0.24
HB × BGase		0.46	0.29	0.34	0.73	0.003	0.70	0.71	0.28	0.46	0.24	0.78	0.95	0.001	0.59	0.87	0.42	0.88

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

[1]HB - hulless barley; BGase - β-glucanase; SCFA - short chain fatty acids; Ace - Acetic acid; Pro - Propionic acid; Buty - Butyric acid; Isob - Isobutyric acid; Val - Valeric acid; Isov - Isovaleric acid; Cap - Caproic acid; Lac - Lactic acid.

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

Table 3. 6. Effects of hulless barley and β-glucanase on caecal short chain fatty acids of broiler chickens aged 28 days

HB[1] (%)	BGase (%)	SCFA µmol/g of wet caecal content								Molar percentage of total SCFA						
		Total	Ace	Pro	Buty	Isob	Val	Isov	Cap	Ace	Pro	Buty	Isob	Val	Isov	Cap
	0	283.9	168.8	58.6	26.6	8.7	8.6	8.6	3.7	59.3	20.6	9.5	3.0	3.0	3.0	1.3
0	0.01	285.0	167.5	59.4	27.8	8.8	8.7	8.7	3.7	58.8	20.7	9.7	3.0	3.0	3.0	1.3
	0.1	281.0	166.5	58.1	26.7	8.6	8.5	8.6	3.7	59.2	20.6	9.5	3.0	3.0	3.0	1.3
	0	269.4	159.1	55.5	25.4	9.3	8.1	8.2	3.5	59.0	20.5	9.5	3.5	3.0	3.0	1.3
30	0.01	248.1	148.7	48.9	24.5	7.6	7.4	7.5	3.2	60.0	19.5	9.9	3.0	3.0	3.0	1.3
	0.1	277.9	164.0	57.6	27.3	8.5	8.4	8.1	3.6	58.9	20.7	9.8	3.0	3.0	2.9	1.3
	0	284.1	166.7	58.3	28.0	9.9	8.6	8.6	3.7	58.7	20.5	9.8	3.5	3.0	3.0	1.3
60	0.01	282.9	166.6	58.5	27.9	8.7	8.6	8.6	3.7	58.9	20.6	9.8	3.0	3.0	3.0	1.3
	0.1	274.4	162.0	56.5	27.1	8.4	8.3	8.3	3.5	59.0	20.6	9.9	3.0	3.0	3.0	1.3
SEM[2]		4.70	2.76	1.05	0.45	0.21	0.15	0.15	0.06	0.10	0.10	0.05	0.06	0.01	0.01	0.003
Main effects																
HB (%)																
0		283.3	167.6	58.7	27.0	8.7	8.6	8.6	3.7	59.1	20.6	9.6	3.0	3.0	3.0	1.3
30		272.0	157.3	54.0	25.8	8.5	8.0	7.9	3.4	59.3	20.2	9.7	3.2	3.0	3.0	1.3
60		277.8	165.1	57.8	27.7	9.0	8.5	8.5	3.6	58.8	20.5	9.8	3.2	3.0	3.0	1.3
BGase (%)																
0		279.1	164.9	57.5	26.7	9.3	8.4	8.5	3.6	59.0	20.5	9.6	3.3	3.0	3.0	1.3
0.01		272.0	161.0	55.6	26.7	8.4	8.2	8.3	3.5	59.2	20.3	9.8	3.0	3.0	3.0	1.3
0.1		277.8	164.2	57.4	27.0	8.5	8.4	8.3	3.6	59.1	20.6	9.7	3.0	3.0	3.0	1.3
Probability																
HB		0.23	0.27	0.14	0.21	0.60	0.23	0.15	0.24	0.16	0.23	0.13	0.59	0.86	0.47	0.80
BGase		0.80	0.82	0.71	0.94	0.18	0.82	0.89	0.82	0.54	0.37	0.27	0.13	0.63	0.57	0.63
HB × BGase		0.67	0.78	0.42	0.58	0.56	0.63	0.84	0.63	0.07	0.14	0.71	0.69	0.96	0.73	0.96

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

[1]HB - hulless barley; BGase - β-glucanase; SCFA - short chain fatty acids; Ace - Acetic acid; Pro - Propionic acid; Buty - Butyric acid; Isob - Isobutyric acid; Val - Valeric acid; Isov - Isovaleric acid; Cap - Caproic acid; Lac - Lactic acid.

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

Table 3. 7. Effects of hulless barley and β-glucanase on gastro-intestinal pH of broiler chickens aged 28 days

Hulless barley (%)	BGase[1] (%)	Crop	Gizzard	Duodenum	Jejunum	Ileum	Caeca	Colon
	0	5.08	3.33	6.09	5.93	6.73	5.85	6.63
0	0.01	5.41	3.58	6.05	5.99	6.98	6.09	6.56
	0.1	5.08	3.51	6.07	6.01	6.87	5.75	6.52
	0	5.56	3.33	6.06	5.91	7.05	5.99	6.99
30	0.01	5.30	3.20	6.09	5.95	6.98	5.83	6.88
	0.1	5.18	3.48	6.18	5.96	7.08	5.77	6.69
	0	5.29	3.54	6.05	5.99	7.08	6.02	6.92
60	0.01	4.87	3.50	6.16	5.97	7.12	5.84	6.98
	0.1	5.23	3.26	6.19	6.01	7.26	6.04	7.17
SEM[2]		0.059	0.043	0.018	0.014	0.033	0.035	0.048
Main effects								
Hulless barley (%)								
0		5.19	3.47	6.07	5.98	6.86[b]	5.90	6.57[b]
30		5.35	3.34	6.11	5.94	7.03[a]	5.86	6.85[a]
60		5.13	3.43	6.13	5.99	7.15[a]	5.97	7.02[a]
BGase (%)								
0		5.31	3.40	6.07	5.94	6.95	5.95	6.85
0.01		5.19	3.43	6.10	5.97	7.02	5.92	6.80
0.1		5.16	3.42	6.15	5.99	7.07	5.85	6.79
Probability								
Hulless barley		0.25	0.31	0.34	0.34	0.0004	0.45	0.0003
BGase		0.52	0.94	0.19	0.27	0.26	0.50	0.88
Hulless barley × BGase		0.11	0.06	0.49	0.84	0.37	0.07	0.36

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

3.4.6 Digestive tract morphology

Hulless barley and BGase interaction was significant on the empty weights of the crop, gizzard, duodenum, full small intestine, and colon, however the differences were consistant with less clear trends (Table 3.8). The crop weight was higher with 0 compared to 0.01% BGase when

the birds were fed 60% HB. The gizzard weight was higher with 0.01 compared to 0% BGase for the 60% HB fed birds. The empty jejunum weight was higher at 0 compared to 30% HB, and the empty weight of the ileum was higher at 60 compared to 0 and 30% HB. Hulless barley and BGase interaction was found for the lengths of the duodenum and colon, and both lengths were increased with increasing HB. In addition, the lengths of the jejunum, ileum and caeca were higher at 60 compared to 0% HB and were higher or equal compared to 30% HB. An interaction of the main effects was also found on the length of the full small intestine, but with minor differences (Table 3.8). It was higher with 0 compared to 0.01 and 0.1% BGase when the birds were given wheat-based diets. Hulless barley and BGase effects were significant on various organ weights and GI content weights (Table 3.9). There was an interaction of BGase and HB levels on the gizzard content, and it increased with the increasing level of HB in the diets containing 0.01% BGase. Further, it was lower with 0 compared to 0.01 and 0.1% BGase for the 60% HB diets. Both duodenum and jejunum contents were lower at 30 and 60% HB levels compared to 0% HB, and the jejunum content decreased with the increasing levels of BGase. Both ileum and colon contents were higher at 60 compared to 0 and 30% HB. The small intestine content was higher at 60 compared to 30% HB, whereas the content decreased with the increasing level of BGase. The pancreas weight increased with the increasing level of HB, whereas the pancreas weight decreased at the level of 0.1 compared to 0.01% BGase.

Table 3. 8. Effects of hulless barley and β-glucanase on gastro-intestinal tissue weights and lengths (proportional to body weight) of broiler chickens aged 28 days

HB[1] (%)	BGase (%)	Empty weight									Length					
		Crop	Proven	Gizzard	Duo	Jejunum	Ileum	SI	Caeca	Colon	Duo	Jejunum	Ileum	SI	Caeca	Colon
	0	0.28^{b}	0.40	1.14^{cd}	0.75^{ab}	1.40	0.91	3.05^{ab}	0.35	0.13^{cd}	1.67^{b}	4.09	3.89	9.95^{ab}	1.58	0.38^{b}
0	0.01	0.28^{b}	0.40	1.09^{cd}	0.72^{ab}	1.29	0.84	2.84^{ab}	0.33	0.12^{d}	1.66^{b}	3.70	3.58	8.93^{c}	1.55	0.34^{b}
	0.1	0.30^{ab}	0.37	1.06^{d}	0.74^{ab}	1.37	0.91	3.01^{ab}	0.37	0.13^{cd}	1.69^{b}	3.92	3.79	9.39^{bc}	1.59	0.38^{b}
	0	0.31^{ab}	0.38	1.24^{abc}	0.73^{ab}	1.28	0.93	2.94^{ab}	0.36	0.14^{cd}	1.74^{ab}	4.07	3.98	9.78^{abc}	1.66	0.41^{ab}
30	0.01	0.28^{b}	0.38	1.19^{bcd}	0.71^{b}	1.29	0.90	2.89^{ab}	0.37	0.13^{cd}	1.74^{ab}	4.03	4.01	9.76^{abc}	1.69	0.37^{b}
	0.1	0.28^{b}	0.38	1.24^{abc}	0.73^{ab}	1.25	0.85	2.83^{b}	0.39	0.14^{cd}	1.81^{ab}	3.93	3.96	9.70^{abc}	1.69	0.40^{ab}
	0	0.34^{a}	0.38	1.19^{bcd}	0.72^{ab}	1.37	1.00	3.03^{ab}	0.36	0.17^{ab}	1.72^{ab}	4.21	4.18	10.25^{ab}	1.67	0.41^{ab}
60	0.01	0.28^{b}	0.40	1.38^{a}	0.80^{a}	1.35	0.98	3.13^{a}	0.39	0.18^{a}	1.95^{a}	4.39	4.32	10.64^{a}	1.83	0.45^{a}
	0.1	0.29^{ab}	0.38	1.32^{ab}	0.73^{ab}	1.30	0.91	2.94^{ab}	0.37	0.15^{bc}	1.75^{ab}	4.01	4.11	10.07^{ab}	1.69	0.39^{ab}
SEM[2]		0.003	0.005	0.015	0.007	0.012	0.010	0.019	0.006	0.002	0.017	0.042	0.041	0.077	0.017	0.005
Main effects																
HB (%)																
0		0.29	0.39	1.10	0.74	1.35^{a}	0.89^{b}	2.97	0.35	0.13	1.67	3.90^{b}	3.76^{c}	9.42	1.57^{b}	0.37
30		0.29	0.38	1.22	0.72	1.28^{b}	0.89^{b}	2.89	0.37	0.14	1.76	4.01^{ab}	3.98^{b}	9.75	1.68^{a}	0.39
60		0.31	0.39	1.30	0.75	1.34^{ab}	0.97^{a}	3.03	0.37	0.16	1.81	4.21^{a}	4.21^{a}	10.32	1.73^{a}	0.42
BGase (%)																
0		0.31	0.39	1.19	0.73	1.35	0.95	3.01	0.36	0.15	1.71	4.13	4.02	9.99	1.64	0.40
0.01		0.29	0.39	1.22	0.74	1.31	0.91	2.95	0.37	0.14	1.78	4.04	3.97	9.78	1.69	0.39
0.1		0.29	0.38	1.20	0.73	1.31	0.89	2.93	0.38	0.14	1.75	3.95	3.95	9.72	1.66	0.39
Probability																
HB		0.16	0.82	0.0001	0.23	0.01	0.002	0.01	0.17	0.0001	0.004	0.01	0.0001	<.0001	0.0005	0.0003
BGase		0.005	0.57	0.52	0.86	0.22	0.07	0.23	0.40	0.12	0.24	0.21	0.77	0.26	0.49	0.63
HB × BGase		0.04	0.88	0.01	0.04	0.21	0.19	0.04	0.43	0.002	0.03	0.10	0.26	0.02	0.27	0.02

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

[1]HB - hulless barley; BGase - β-glucanase; Proven – proventriculus; Duo - duodenum; SI - small intestine.

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

Table 3. 9. Effects of hulless barley and β-glucanase on gastro-intestinal content and organ weights as a percentage of body weight of broiler chickens aged 28 days

HB[1] (%)	BGase (%)	Content									Weight		
		Crop	Provent	Gizzard	Duodenum	Jejunum	Ileum	SI	Caeca	Colon	Liver	Spleen	Pancreas
	0	0.64	0.07	0.89^{bc}	0.11	0.98	0.80	1.88	0.26	0.13	2.65	0.11	0.22
0	0.01	0.52	0.09	0.65^{c}	0.10	0.89	0.80	1.78	0.27	0.12	2.51	0.11	0.21
	0.1	0.42	0.04	0.73^{bc}	0.13	0.82	0.83	1.79	0.26	0.13	2.51	0.10	0.20
	0	0.56	0.05	1.00^{abc}	0.09	0.93	0.82	1.83	0.31	0.15	2.42	0.10	0.22
30	0.01	0.37	0.05	1.14^{ab}	0.07	0.79	0.80	1.65	0.32	0.13	2.56	0.11	0.24
	0.1	0.41	0.03	1.06^{abc}	0.09	0.64	0.75	1.47	0.25	0.12	2.41	0.10	0.22
	0	0.40	0.03	0.93^{bc}	0.09	1.04	1.17	2.29	0.30	0.19	2.40	0.10	0.24
60	0.01	0.71	0.05	1.46^{a}	0.06	0.89	1.06	2.01	0.26	0.19	2.57	0.10	0.26
	0.1	0.52	0.03	1.14^{ab}	0.07	0.74	0.90	1.69	0.24	0.16	2.50	0.09	0.23
SEM[2]		0.054	0.005	0.037	0.004	0.020	0.023	0.038	0.010	0.005	0.020	0.002	0.003
Main effects													
HB (%)													
0		0.53	0.06	0.76	0.11^{a}	0.90^{a}	0.81^{b}	1.82^{ab}	0.26	0.13^{b}	2.56	0.11	0.21^{c}
30		0.45	0.04	1.07	0.08^{b}	0.79^{b}	0.79^{b}	1.65^{b}	0.30	0.13^{b}	2.47	0.10	0.23^{b}
60		0.54	0.04	1.17	0.07^{b}	0.79^{b}	1.04^{a}	2.00^{a}	0.27	0.18^{a}	2.49	0.10	0.25^{a}
BGase (%)													
0		0.53	0.05	0.94	0.10	0.98^{a}	0.93	2.00^{a}	0.29	0.15	2.49	0.10	0.23^{ab}
0.01		0.53	0.06	1.08	0.08	0.86^{b}	0.89	1.81^{ab}	0.29	0.15	2.55	0.11	0.24^{a}
0.1		0.45	0.03	0.98	0.10	0.73^{c}	0.83	1.65^{b}	0.25	0.14	2.48	0.10	0.22^{b}
Probability													
HB		0.65	0.20	<.0001	<.0001	0.02	<.0001	0.0006	0.41	<.0001	0.13	0.34	<.0001
BGase		0.67	0.13	0.21	0.40	<.0001	0.15	0.0005	0.25	0.35	0.25	0.46	0.001
HB × BGase		0.41	0.77	0.009	0.34	0.73	0.21	0.24	0.73	0.57	0.06	0.70	0.45

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

3.4.7 Performance parameters

There were significant effects of HB and BGase on the performance parameters in different periods of the experiment (Table 3.10). Interactions of HB and BGase were found for BWG at all the time periods except d 21-28 broiler chickens. Overall, BWG decreased with increasing HB when considering all the time periods. For the 0 and 30% HB levels, BGase did not affect the BWG in any of the time periods. However, at the 60% HB, BWG was lower with 0.01 and 0.1 compared to 0% BGase in the birds aged d 0-7. However, after d 7, there was no BGase effect on BWG at the 60% HB except at the time period of d 0-28, as the BWG was lower with 0.01 compared to 0% BGase.

Feed intake at d 0-7 was lower at 0.1 compared to 0 and 0.01% BGase in the diets. The interaction between HB and BGase was found for the FI at d 14-21 and the total period. However, BGase did not affect FI at each HB level except for the FI at d 0-28 as the FI was lower at the 0.01 compared to 0.1% BGase when the birds were given 60% HB-based diets.

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

Table 3. 10. Effects of hulless barley and β-glucanase on production performance of broiler chickens

HB[1] (%)	BGase (%)	BWG (kg)					FI (kg)					F:G				
		d 0-7	d 7-14	d 14-21	d 21-28	d 0-28	d 0-7	d 7-14	d 14-21	d 21-28	d 0-28	d 0-7	d 7-14	d 14-21	d 21-28	d 0-28
	0	140^{a}	306^{a}	532^{ab}	704	1682^{a}	167	400	722^{ab}	1032	2320^{ab}	1.19^{b}	1.31^{c}	1.36	1.47	1.39
0	0.01	143^{a}	318^{a}	542^{a}	720	1723^{a}	165	409	730^{ab}	1039	2342^{ab}	1.15^{b}	1.29^{c}	1.35	1.45	1.36
	0.1	135^{ab}	311^{a}	525^{ab}	705	1676^{a}	157	399	704^{ab}	1010	2254^{b}	1.16^{b}	1.28^{c}	1.34	1.43	1.35
	0	138^{a}	297^{ab}	521^{ab}	685	1645^{ab}	162	393	713^{ab}	1007	2256^{ab}	1.17^{b}	1.31^{c}	1.38	1.48	1.40
30	0.01	140^{a}	314^{a}	537^{a}	690	1680^{a}	165	419	733^{a}	1024	2325^{ab}	1.18^{b}	1.34^{bc}	1.37	1.49	1.39
	0.1	135^{ab}	309^{a}	525^{ab}	691	1659^{ab}	158	403	715^{ab}	1004	2280^{ab}	1.17^{b}	1.31^{c}	1.36	1.46	1.38
	0	143^{a}	303^{ab}	507^{abc}	698	1649^{ab}	167	421	729^{ab}	1055	2371^{a}	1.17^{b}	1.39^{ab}	1.44	1.53	1.45
60	0.01	127^{b}	280^{b}	473^{c}	655	1535^{c}	160	404	687^{b}	1004	2254^{b}	1.26^{a}	1.45^{a}	1.45	1.53	1.47
	0.1	126^{b}	296^{ab}	498^{bc}	656	1575^{bc}	157	403	705^{ab}	1004	2284^{ab}	1.26^{a}	1.35^{bc}	1.42	1.54	1.43
SEM[2]		1.03	2.20	3.58	4.57	9.22	0.93	2.46	3.72	5.27	2.46	0.01	0.01	0.01	0.01	0.01
Main effects																
HB (%)																
0		140	312	533	710^{a}	1694	163	403	719	1027	2311	1.17	1.29	1.35^{b}	1.45^{b}	1.37^{c}
30		138	307	528	688^{ab}	1662	162	405	720	1012	2297	1.17	1.32	1.37^{b}	1.47^{b}	1.39^{b}
60		132	293	493	670^{b}	1586	161	408	707	1021	2292	1.23	1.40	1.44^{a}	1.53^{a}	1.45^{a}
BGase (%)																
0		140	305	520	696	1659	165^{a}	405	721	1031	2316	1.18	1.34	1.39	1.49	1.42^{a}
0.01		137	304	517	688	1646	163^{a}	411	717	1022	2312	1.20	1.36	1.39	1.49	1.41^{a}
0.1		132	302	516	684	1637	157^{b}	400	708	1006	2272	1.19	1.31	1.37	1.48	1.39^{b}
Probability																
HB		0.0008	0.0003	0.0001	0.0009	0.0001	0.71	0.62	0.22	0.46	0.69	0.0001	0.0001	0.0001	0.0001	0.0001
BGase		0.0004	0.75	0.85	0.51	0.48	0.002	0.23	0.28	0.12	0.09	0.03	0.006	0.21	0.40	0.002
HB × BGase		0.001	0.006	0.02	0.13	0.004	0.35	0.05	0.02	0.19	0.05	0.0001	0.04	0.82	0.76	0.15

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

3.5 Discussion

Beta-glucan MW distribution in the soluble ileal digesta was assessed to determine the effect of exogenous BGase on depolymerizing high MW β-glucan in broiler chickens. The ileal Mw did not significantly change with the increasing level of HB in the diets, although the interaction between the main effects were significant. In addition, Mp and MW-10% was lower in the treatments with HB compared to wheat. This contrasted with the concept of HB contains high MW β-glucan compared to wheat in general (Biliaderis and Izydorczyk, 2007). The low MW β-glucan of HB in the current study might be due to the activation of endogenous enzymes present in HB due to high moisture containing weather conditions, or storage. It is a well-established fact that water treatment improves the nutritive value of grains including barley (Fry et al., 1958; Lepkovsky and Furuta, 1960) possibly by activating endogenous non-starch polysaccharidases, which results in a lower content of water-soluble pentosans including β-glucan. The endogenous enzyme activation in the grain is further supported by the very low MW values of the ileal digesta in the current study compared to the MW of barley grain (Wang et al., 2016) when the birds were fed HB-based diet, even without adding BGase. Therefore, HB used in the current study might consisted of β-glucan that has lower MW despite the high content of total β-glucan (8.7%), and it is possibly due to the effect of environmental factors or the chemical characteristics of grain.

However, BGase decreased all three MW parameters in the ileal digesta of chickens since BGase depolymerizes high MW β-glucan in the digestive tract of broiler chickens. The effect of BGase on MW-10% was significant at all three HB levels in the diet, and MW-10% is the most important MW criteria in the current research since it demonstrates the increased proportion of low MW β-glucan, which might have the potential to increase carbohydrate fermentation. In addition, the enzyme dosage effect on Mp, and Mw at 30% HB confirms an increased level of BGase (0.1% BGase) breakdown β-glucan into further smaller molecules compared to the standard level of BGase (0.01% BGase). The ileal viscosity was lower with the addition of BGase, which is in accordance to the β-glucan MW distribution data in the ileal digesta of broilers. Further, the degree of BGase associated Mw reduction was higher in HB-based treatments compared to wheat-based diets. It might be associated with the differences in β-glucan

structure among different grains. The differences in the ratio of cellotriosyl to cellotetraosyl units (DP3/DP4) in wheat (3.0-4.5) and barley (2.3-3.4) β-glucan, which results in higher tri-saccharides and lower tetra-saccharides proportions in β-glucan of wheat compared to barley (Biliaderis and Izydorczyk, 2007). Increased aggregation and lower solubility of β-glucan has been reported in β-glucan containing high and low DP3/DP4 (Burton and Fincher, 2014), and it might be the reason for β-glucan showing a lower susceptibility to exogenous BGase in wheat compared to barley.

Beta-glucan digestibility is expected to be low in the small intestine due to the absence or lack of endogenous non-starch polysaccharidases, and there is no information available in the literature regarding β-glucan digestibility in chickens. However, very high and complete β-glucan digestion in the ileum (77-85%) and total tract (100%) was observed in pigs given barley-based diets without addition of BGase (Fadel et al., 1988; Bach Knudsen and Hansen, 1991). The current experiment confirmed β-glucan digestibility in the ileum and total tract were considerably lower in chickens compared to pigs according to previous research. It might be due to the small GIT especially the small caeca in chickens which gives a less capacity to enter fermentable substrate and undergoes bacterial fermentation, and short colon which minimizes fermentation capacity and time availability. Further, high feed passage rate of chickens permits less time to digest fibre in comparison to pigs (Svihus et al., 2002; Kim et al., 2007). The ileal and total tract β-glucan digestibility in the current study was higher when the birds were fed HB- compared to wheat-based diets, and it might be associated with pre-harvest sprouting that activates endogenous BGase in HB. The increased dose of BGase increased β-glucan digestibility with a wheat-based diet even though wheat contains a low amount of β-glucan. However, both ileal and total tract β-glucan digestibility did not increase with BGase addition to a HB-based diet, which is unexpected. Beta-glucan in wheat is thought to be less susceptible to BGase compared to HB, due to the differences in the ratio of cellotriosyl to cellotetraosyl units (DP3/DP4) in wheat (3.0-4.5) and barley (2.3-3.4) β-glucan (Biliaderis and Izydorczyk, 2007). However, β-glucan digestibility in wheat and HB were opposite to the aforementioned concept. Beta-glucanase appeared to decrease the ileal carbohydrate fermentation of chickens fed barley-based diets (Chapter 4, 5 and 6), which supports the diminished BGase effect on β-glucan

digestibility in HB compared to wheat. However, the difference between total tract and ileal β-glucan digestibility data indicates a limited quantity of β-glucan has entered the caeca. Further, a significant portion of the β-glucan has been utilized by the gut microbiota by the time the digesta reaches the ileum.

Nitrogen corrected AME values for both wheat and HB were lower than in general, and it might be due to the different growing conditions and year of the grains that affects grain nutrient content (Bedford et al., 1998; Scott et al., 1998; Ball et al., 2013). However, AME_n decreased with the addition of HB into the diets since HB contains more fibre compared to wheat (Biliaderis and Izydorczyk, 2007; Dhingra et al., 2012) that results in lower energy values. Further, HB and wheat consisted of 29.0% and 15.2% of TDF, respectively in the current study. Overall, BGase increased AME_n of broiler chickens fed a HB-based diet, and it is significant at 60% HB level. It might be due to the increased energy derived from increased digestibility of nutrients that associated with low GIT viscosity, which resulted from the depolymerization of high MW β-glucan (Classen et al., 1985). However, the ileal viscosity in the current study was not higher in birds fed HB- than wheat-based diets, even though HB contains a very high percentage of TDF (29.0%) including β-glucan (8.7%). Further, soluble dietary fibre was also high in HB compared to wheat (9.4 vs 1.6%) in the current research. Moreover, the grain viscosity was high in HB (49.3 cP) compared to wheat (1.7 cP) however, *in vitro* viscosity of ingredients does not always reflect by the viscosity in the digestive tract (Dikeman et al., 2006). The smaller difference of viscosity might be due to the endogenous BGase activation of HB in the field or storage due to high moisture conditions (Fry et al., 1958; Lepkovsky and Furuta, 1960), which results in low MW β-glucan that attributed to a low viscosity in the ileum of broiler chickens. Furthermore, a high level of arabinoxylan in wheat also increases the ileal viscosity in wheat fed broilers (Choct and Annison, 1992; Kiarie et al., 2014). Moreover, the soluble β-glucan content which is the main component that affects viscosity was not analyzed in the current study, although it is generally higher in barley than wheat (Henry, 1985). Nevertheless, there was an enzyme response on the ileal viscosity as the addition of an increased level of BGase breakdowns high MW soluble β-glucan into low MW material despite the low β-glucan content (0.68%). In addition to the decline of digesta viscosity, nutrient digestibility increases

with the elimination of nutrient encapsulation (Hesselman and Åman, 1986) due to the activity of non-starch polysaccharidases in the diets. Apparent metabolizable energy increased with BGase in the current study might be associated with nutrient encapsulation. The increased AME_n with a high level of BGase might be due to increased feed passage rate that results from the reduction of soluble β-glucan, and viscosity of digesta in broiler chickens. Mean retention time in GIT of chickens decrease with the dietary addition of non-starch polysaccharidases (Danicke et al., 1999), and lower mean retention time is associated with high nutrient digestibility in poultry.

Short chain fatty acid levels and gastro-intestinal pH was assessed to investigate the treatment effects on carbohydrate fermentation in broiler chickens since the current study hypothesized improved carbohydrate fermentation with the increasing level of low MW β-glucan in the ileal digesta. Ileum and colon pH were higher with both levels of HB compared to wheat, which is not expected because increased HB content in diets supposed to results in more substrate for fermentation in entire lower GIT causing lower intestinal pH. Further, there was no treatment effect on the caecal pH. However, AME_n decreased with HB in broiler chickens, and it might lead to increased pH in the ileum and colon due to the less availability of substrates especially carbohydrates for fermentation. In addition, the pancreas weight increased with increasing BGase and it might be associated with high pH in the ileum because of the alkaline pancreatic secretions. Nevertheless, intestinal pH is not only an indication of SCFA concentration, but it might also be influenced by ingredient composition in diets including minerals and proteins, and endogenous secretions in chicken GIT. Protein fermentation in lower GIT results in ammonia, biogenic amines, indoles and phenols other than SCFA, which results in increased pH in the GIT content in chickens (Apajalahti, 2005). Increased minerals in feed ingredients are hydrolyzed in GIT and neutralize or buffers the hydrogen ions that resulted from SCFA in digesta, and results in increased pH in the GIT content of chickens (Heller and Penquite, 1936). Further, there were no treatment effects on the ileal and caecal SCFA concentrations of broiler chickens. It agrees with previous research based on barley feeding in broilers, which observed less and inconsistent dietary BGase effects (Józefiak et al., 2005; 2006). The ileal and caecal concentrations of SCFA depends on many factors including the availability of fermentable substrates, SCFA production, and the absorption that depends on the mechanism

of SCFA transport, and the expression of transporters involved with the mechanisms (Tan et al., 2014a). Therefore, SCFA concentrations could be varied despite BGase derived high amount of lower MW carbohydrates, and high fibre content (TDF, SDF and total β-glucan) in HB- in comparison to wheat-based diets in the current study.

It has been observed the effect of SCFA on increasing the production of PYY and GLP-1 from L-cells in the small intestine of mammals (Longo et al., 1991; Keenan et al., 2006; Zhou et al., 2008; Singh et al., 2012; Brooks et al., 2017), and it helps in increasing satiety, and decreases the gastric emptying, GI motility and secretion by activating ileal brake (Meyer et al., 1998; Maljaars et al., 2008). Further, high β-glucan content increased the secretion of PYY and GLP-1 in conventional mice compared to germ-free mice, which indicates the effect of SCFA on GI hormone release (Miyamoto et al., 2018). Hulless barley in comparison to wheat generally increased the relative empty weights, lengths, and content weights of the digestive tract sections in the current study. However, HB induced changes in GI morphological measurements are difficult to relate with the secretion of hormones including PYY and GLP-1, since there were no treatment effects on SCFA levels in the current research.

The high fibre content in HB increases digesta viscosity, and affects nutrient digestibility in broiler chickens (Pettersson and Åman, 1989; Bedford et al., 1991; Choct and Annison, 1992). The total dietary fibre content and total β-glucan were higher in HB than wheat in the current study (TDF; 29 and 15.2%, β-glucan; 8.70 and 0.68%), but significant differences were not observed in the ileal viscosity. Non-starch polysaccharides have the ability to entrap nutrients using endosperm cell walls to limit the access of digestive enzymes, and results in lower nutrient digestibility (Kocher et al., 2003; Khadem et al., 2016). Therefore, the digestive tract increases its' size to increase digestion as a compensatory mechanism. The pancreas weight increased with HB as a compensatory mechanism to increase the efficiency of nutrient digestion by increasing secretion of digestive enzymes, since soluble fibre especially β-glucan in HB negatively affects digestion of other nutrients. However, the pancreas weight decreased with BGase since nutrient digestibility increases with BGase addition, thus the requirement of digestive enzymes decreases in broiler chickens.

The gizzard content increased with the addition of HB to the diets, and it demonstrates the necessity of increased gizzard retention time of digesta to complete the grinding of high fibre content in HB. The content weights of the duodenum and jejunum decreased with HB, and it might be associated with high insoluble fibre in HB, which increase digesta passage rate (Hetland & Svihus, 2001). However, HB increased the ileum and caeca content weights, and it is probably due to the solubilization of insoluble fibre, which increases feed retention time in the lower digestive tract of broiler chickens (Almirall and Esteve-Garcia, 1994; Salih et al., 1991). Beta-glucanase effect was significant on the jejunum and small intestine content, and the reduction of the content is associated with the depolymerization of β-glucan that reduces digesta viscosity and thereby increases feed passage rate (Almirall and Esteve-Garcia, 1994).

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

In conclusion, exogenous BGase depolymerized high MW β-glucan in the digestive tract of broiler chickens, and there was a significant enzyme dosage effect which demonstrates the beneficial effect of increased dose of BGase in comparison to the standard level of BGase used in the feed industry. The resulting low MW β-glucan was not able to increase the carbohydrate fermentation in the ileum and caeca however, HB and BGase affected the digestive tract characteristics and production performance of broiler chickens.

4.0 EFFECTS OF DIET HULLESS BARLEY AND BETA-GLUCANASE LEVELS ON PERFORMANCE, ILEAL DIGESTA SOLUBLE BETA-GLUCAN MOLECULAR WEIGHT AND DIGESTIVE TRACT CHARACTERISTICS OF BROILERS CHALLENGED FOR COCCIDIOSIS

4.1 Abstract

Diet β-glucanase (BGase) depolymerizes viscous β-glucan into lower molecular weight (MW) 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 performance, β-glucan depolymerization, and digestive tract characteristics. 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 x 3 factorial arrangement. A total of 5346 broilers were raised in litter floor pens and vaccinated for coccidiosis in feed and water on d 5. Each treatment was assigned to 1 pen (66 birds) in each of 9 rooms. Overall, HB decreased body weight gain (BWG) and increased feed: gain (F:G) of broilers. From d 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, while the 0.1% BGase had no effect on BWG, and increased F:G. For the d 22-32 and 0-32 time 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. At both 11 and 33 d, the peak MW of β-glucan in ileal digesta decreased with increasing BGase for 30 and 60% HB. The maximum MW for the 10% smallest β-glucan molecules (MW-10%) decreased with BGase at both ages for 30 and 60% HB; for birds fed 0% HB, 0.1% BGase decreased MW-10%. Interactions were found for ileal minor SCFA (without clear trends) at d 11 and 33. The 0.1% BGase increased all caecal SCFA compared to the 0.01% BGase at d 11 only for the 60% HB. Ileal pH increased with increasing HB and BGase at d 11 and 33. Caecal pH was lower for 0.1 than 0% BGase for 60% HB at d 11. Overall, HB increased digestive tract size and content, whereas BGase reduced them. In conclusion, BGase depolymerized high MW β-glucan in HB in a dose-dependent

manner, resulting in reduced Mp and a higher proportion of small MW β-glucan. The data suggest that producing high levels of ileal low MW β-glucan decreases and increases performance of young and old broilers, respectively.

Keywords: prebiotics, non-starch polysaccharides, fermentation, SCFA, pH

4.2 Introduction

The use of antibiotics in poultry feed has decreased in recent years, with the change mostly driven by regulatory agencies (European Commission, 2005; Chicken Farmers of Canada, 2019) and voluntary reduction by poultry production companies in response to consumer demand (Goddard et al., 2017; Karavolias et al., 2018). The change in legislature and consumer attitude relates to the potential for antibiotic residues in poultry products (Furtula et al., 2010; Gonzalez Ronquillo and Angeles Hernandez, 2017), and the development of antibiotic-resistant genes in pathogenic bacteria (Diarra et al., 2007; Furtula et al., 2010; Kaesbohrer et al., 2012; Garcia-Migura et al., 2014; Roth et al., 2019), that in turn impact food safety and public health (Smith et al., 2002; Marshall and Levy, 2011). A negative consequence of reduced antibiotic use in poultry production is the increased incidence of enteric disease, which results in economic losses in the poultry industry.

The reduction in use of in-feed antibiotics has made investigation of alternatives to antibiotics a major research priority. Probiotics, prebiotics, essential oils, volatile fatty acids (eg: butyric acid) and feed enzymes (eg: non-starch 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). To fully understand the efficacy of alternatives, testing in chickens undergoing a disease challenge is important so that their ability to alleviate negative infection effects can be more clearly delineated.

Prebiotics have been extensively studied as an alternative strategy to mitigate the negative effects of reduced antibiotic use in the poultry industry. Recently the International Scientific Association of Probiotics and Prebiotics defined a prebiotic as “a substrate that is selectively utilized by host microorganisms conferring a health benefit” (Gibson et al., 2017).

Prebiotics contribute to improved digestive tract characteristics and production performance in poultry through different mechanisms including competitive exclusion of pathogenic bacteria by competing for the sites of attachment in the host, providing substrates for fermentation (Ofek and Beachey, 1978; Baurhoo et al., 2007; Rebole et al., 2010; Kim et al., 2011; Pourabedin et al., 2014; 2017; Corrigan et al., 2015), improving gastro-intestinal (GI) morphological structure (Xu et al., 2003; Baurhoo et al., 2007; 2009; Chee et al., 2010; Pourabedin et al., 2014; Shang et al., 2015; Ding et al., 2018), producing anti-microbial factors (Chen et al., 2007; Munoz et al., 2012) and modulating the host immune system (Babu et al., 2012; Yitbarek et al., 2012; Huang et al., 2015).

Commonly studied prebiotics in chickens are fructo-oligosaccharides, mannan-oligosaccharides, arabinoxylo-oligosaccharides (AXOS) and xylo-oligosaccharides (XOS). A common characteristic of prebiotics is that they are not digestible by chickens and as a result are fermentable in the lower gastro-intestinal tract (GIT). Because of fermentation and the production of fermentation products, they exert prebiotic properties by modifying the GI microbial population and epithelial integrity and stimulating the immune system by modulating cytokine production (Pourabedin and Zhao, 2015). Most prebiotics tested in broiler diets are fed in extracted form (often oligosaccharides) at specific doses (2-10 g/kg), but potential prebiotics also exist in common feed ingredients. For example, feeding AXOS and/or XOS in wheat-based diets has resulted in modification of GIT microbiota (Courtin et al., 2008b; De Maesschalck et al., 2015; Pourabedin et al., 2015), increased SCFA production including butyric, acetic and propionic acids (Pourabedin et al., 2015; Keerqin et al., 2017 Ding et al., 2018; Yuan et al., 2018), improved intestinal epithelial morphology (De Maesschalck et al., 2015; Ding et al., 2018) and down-regulation of the pro-inflammatory response in chickens (Yuan et al., 2018). However, these oligosaccharides can also be formed by enzymatic depolymerization of arabinoxylan found in cereal grains and thereby produce similar effects to those found by adding purified oligosaccharides (Masey-O'Neill et al., 2014; Lee et al., 2017). The same principle can be used to project the prebiotic effects of other fibre types such as β-glucan, which is also found in cereal grains.

It is common practice to use exogenous β-glucanase (BGase) in barley-based poultry feed to reduce β-glucan induced digesta viscosity (Salih et al., 1991; Fuente et al., 1995; Yu et al., 2002) and mitigate the negative effects associated with viscosity. In addition to reducing negative effects on digestibility and bird performance, dietary BGase also has been found to modify the microbial population in the GIT of chickens (Kaldhusdal and Hofshagen, 1992; Malthlouthi et al., 2002; Józefiak et al., 2006). In addition, enzyme use has been observed to affect bacterial fermentation (SCFA level) and GI pH, factors thought to affect digestive tract microbial populations (Józefiak et al., 2005, 2006, 2010). However, the effects of exogenous BGase on carbohydrate fermentation in broilers fed barley-based diets (Józefiak et al., 2005; 2006) have not been consistent, possibly due to variation in grain β-glucan characteristics and BGase source (purity, dose). Research with poultry suggests that β-glucan may act as a prebiotic, but the evidence is not definitive.

A prebiotic effect of cereal β-glucan has been demonstrated in mammalian species (Queenan et al., 2007; Shen et al. 2012; Metzler-Zebeli and Zebeli, 2013), and almost complete digestion of cereal β-glucan at the end of the digestive tract has been shown in pigs (Fadel et al., 1988; Bach Knudsen and Hansen, 1991). However, extrapolation of these findings to chickens is ill-advised because of major differences in digestive tract anatomy and bacterial fermentation capacity. Further, information is lacking on the degree of β-glucan depolymerization accomplished by enzyme use in chickens and how this affects fermentation and other digestive tract characteristics. 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 and other digestive tract characteristics in broiler chickens fed a barley-based diet (Dos Santos et al., 2013).

The objective of the study was to evaluate the effects of diet hulless barley (HB) and BGase levels on performance, ileal digesta soluble β-glucan molecular weight (MW) distribution and digestive tract characteristics of broiler chickens under a coccidiosis challenge. It was hypothesized that level of BGase will impact the degree of β-glucan depolymerization and the production of low MW β-glucan. In turn, these changes will increase carbohydrate fermentation and affect other digestive tract characteristics, resulting in increased broiler performance.

4.3 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).

4.3.1 Birds and housing

A total of 5346 one d 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 nine environmentally controlled rooms with an estimated trial end density of 31 kg/m^2. There were nine floor pens in each room. Each of the nine dietary treatments was randomly assigned to one pen per room providing nine replications per treatment. An equal amount of straw was placed in each room with a 7.5-10 cm thickness. Room temperature was 33°C on d 0, and then gradually decreased until it was 21°C by d 25. Day length was 23 h at the beginning of the trial, and it was gradually reduced to 17 h by d 12. Light intensity was 20 lux at the start of the trial and gradually reduced to 10 lux by d 10. Each pen was equipped with a tube feeder having a pan diameter of 36 (0 to 25 d) or 43 cm (>25 d) to provide *ad-libitum* feed. Each pen was provided with a height-adjustable nipple drinker each having six 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 cell).

4.3.2. Experimental diets

Treatments were arranged in a 3 × 3 factorial design based on diet HB (CDC Fibar; 0, 30, 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 d 0 to 11 and grower diets thereafter. The ingredients and calculated nutrient levels are presented in Table 4.1. Diets were formulated according to 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 70-75°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 according to the AB Vista methods of ESC Standard Analytical Method SAM042-01 and SAM038, respectively. Xylanase activity was non-detectable in the diets, and BGase activity approximated the expected enzyme activity values.

Table 4. 1. Ingredients and calculated nutrient levels of starter and grower diets (%)

Ingredient	Starter	Grower
Cereal grain (wheat or hulless barley)[1]	59.09	60.00
Wheat (remaining)	0	4.80
Soybean meal	32.97	26.93
Canola oil	3.29	4.03
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
Nutrient, calculated		
AME (kcal/kg)	3000	3100
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	1.35
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 fibre (TDF) 14.4, insoluble dietary fibre (IDF) 12.4, soluble dietary fibre (SDF) 2.0, total β-glucan 0.64; hulless barley - TDF 26.7, IDF 18.9, SDF 7.8, total 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 3773 mg.

4.3.3. Coccidiosis challenge

All the birds were challenged with Coccivac B-52 live vaccine, which contains *Eimeria acervulina, E. mivati, E. maxima* (two strains) and *E. tenella* oocysts. Vaccination (1.3 × recommended dose) was completed at 5 d of age to facilitate uniform oocyst intake by spraying diluted vaccine (1000 doses in 500 ml distilled water) onto one egg tray containing feed and one 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 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 vaccination 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.

4.3.4 Performance data collection

Performance parameters including feed intake (FI) and body weight were taken on a pen basis at d 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.

4.3.5 Sample collection

There were two sample collection points (d 11 and 33). At each collection day, four birds were selected from each pen, weighed individually and euthanized by injection of T-61 containing embutramide, mebezonium iodide and tetracaine hydrochloride (Merck Animal Health, Kirkland, Quebec, Canada) into the brachial vein. Two birds were used to collect samples for histology, gene expression, SCFA analysis, and pH measurement. Initially, *in situ* pH was measured in the content of the crop, gizzard, duodenum, jejunum, ileum, caeca and colon using a Beckman Coulter 34 pH meter (Model PHI 34, Beckman Instruments, Fullerton, CA). Samples (1 cm) of the ileum (middle section) were removed carefully and placed in 10% neutral buffered formalin for histological evaluation; samples were stored at room temperature until

examination. Samples (about 2 cm) were collected after removing contents from the ileum and caeca (middle sections) into sterile plastic bags and placed in liquid nitrogen, and then stored in -80°C until analysis for gene expression. Entire ileal and caecal contents were also collected into plastic centrifuge tubes and stored at -20°C for the analysis of SCFA. Two birds were used to obtain GIT size and organ weights. Tissues were removed from the bird carcass, separated into different GIT sections (crop, proventriculus, gizzard, duodenum, jejunum, ileum, caeca, 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 measurements. Ileal contents were collected into plastic snap-cap vials and centrifuged for 3-5 min at 14,000 rpm using a Beckman microfuge (Model E348720, Beckmann instruments, INC, Palo Alto, CA). A Brookfield digital viscometer (Model LVDV-III, Brookfield Engineering Labs, INC, Stoughton, MA 02072) was used to measure ileal supernatant viscosity. A fraction of ileal supernatant derived from centrifugation was stored at -80°C for the analysis of β-glucan MW distribution.

4.3.6 Dietary analysis

Experimental diets and ingredients (HB and wheat) were ground using a Retsch laboratory mill (Retsch ZM 200, Germany) to 1 mm (for the analysis of insoluble and soluble dietary fibre, N, fat, ash, amino acids and minerals) and 0.5 mm (for the analysis of total starch and β-glucan) screen-hole sizes. Insoluble and soluble dietary fibre (IDF and SDF) were analyzed using a Megazyme kit (Total dietary fibre assay procedure, Megazyme International Ireland Ltd., Bray Business Park, Bray, Co. Wicklow, Ireland) according to the AOAC method 991.43 and AACC method 32-07.01, and total dietary fibre (TDF) was obtained by addition. Beta-glucan was analyzed (AOAC Method 995.16, AACC Method 32-23 and ICC Standard Method No. 168) 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 and AACC method 76-13.01 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, USA), 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, USA) following the AOAC method 920.39. Experimental diets were analyzed for amino acids by Agriculture Experiment Station Chemical Laboratories (University of Missouri, Columbia) using AOAC method 982.30 E (a, b, c). Ash content was analyzed according to AOAC method 942.05 using a muffle oven (Model Lindberg/Blue BF51842C, Asheville, NC 28804, USA). Moisture was analyzed using AOAC method 930.15. Mineral analysis of the ingredients was performed by SGS Agrifood Laboratories (Guelph, Ontario, Canada) using AOAC method 985.01.

4.3.7 Beta-glucan molecular weight distribution

Ileal supernatant was boiled for 15 min to inactivate endogenous BGase activity in the sample and centrifuged at 10,000 rpm for 5 min using a Beckman microfuge (Model E348720, Beckmann instruments, INC, Palo Alto, CA). Ileal supernatant was analyzed for β-glucan MW using size exclusion chromatography with calcofluor post-column detection for fluorescent recognition (Boyd et al., 2017). The HPLC used two columns (Shodex OHpak SB-806M column with OHpak SB-G guard column and a Waters Ultrahydrogel linear column). The mobile phase was 0.1M Tris buffer (pH=8). Peak molecular weight (Mp) and weight average molecular weight (Mw) were obtained using a molar mass distribution curve. Peak molecular weight is the molecular weight of the most abundant β-glucan proportion. Weight average molecular weight is the average of all the molecular weights of β-glucan (based on weight fraction of each type of molecule). In addition, the maximum molecular weight for the smallest 10% β-glucan molecules (MW-10%) was also assessed based on the molar mass distribution curve.

4.3.8 Short chain fatty acids analysis

Short chain fatty acids were analyzed using the method described by Zhao et al. (2006) with minor modifications. Samples were analyzed in triplicate. The internal standard for gas chromatography was prepared using 20 ml of 25% phosphoric acid, 300 µl of isocaproic acid and deionized water. The standard solution was made up of 300 µl acetic acid, 200 µl propionic acid, 100 µl butyric acid and 50 µl of isobutyric, isovaleric, valeric, caproic and lactic acids. The

digesta is thawed and mixed with 25% phosphoric acid at 1:1. It was kept at room temperature for 10 min with occasional shaking and centrifuged at 12,000 rpm for 10 min. Then 1 ml of supernatant was mixed with 1 ml of the internal standard and centrifuged at 16,000 rpm for 10 min. Then the supernatant was filtered through a 0.45-micron nylon filter. The filtrate was added to a Gas Chromatographic autosampler vial. After that, it was injected into the Zebron Capillary Gas Chromatography column (Zebron^{TM}ZB-FFAP, Phenomenex, Torrance, CA). The column length was 30 m and an internal diameter of 0.25 mm. The film thickness of the column was 0.25 µm. Thermos Scientific Gas chromatography system (Model Trace 1310, Milan, Italy) equipped with a flame ionization detector was used for the analysis.

4.3.9 Histology of gastro-intestinal wall

The 10% formalin buffered saline preserved ileal tissue samples were cut into two longitudinal sections and embedded in paraffin. Two slides were made for each sample. One slide was stained with hematoxylin and eosin to obtain the GIT morphology measurements, whereas the other slide was stained with Alcian Blue/ Periodic Acid-Schiff for differentiation of goblet cells (GC). Villi length and width, and crypt depth were measured in 8-10 well-oriented villi and crypts per section. Slides were observed and images were captured using an Optika B-290TB digital microscope (Bergamo, Italy) with an HDCE-X3 digital camera. Optika vision lite software was used to capture the images. Ileal morphology measurements of captured images were obtained using Scope Image 9.0 professional imaging software (BP Integrated Technologies, Inc, Calamba City, Philippines). Villus length was considered as the length from the tip of a villus to the villus-crypt junction. Villus width was measured at half the height of a villus. Crypt depth was considered as the depth of the invagination between two adjacent villi. Goblet cells were categorized as acidic mucin-producing GC (appears in blue), neutral mucin-producing GC (appears in magenta) and mixed mucin-producing GC (appears in purple) (Osho et al., 2017). Goblet cells were counted around the perimeter of 8-10 well-oriented villi per section.

4.3.10 Gene expression

Ileal and caecal tissue samples, frozen in liquid nitrogen, were homogenized using a mortar and pestle and then stored at -80ºC until RNA extraction. RNA was extracted from the

ground samples according to the user manual of the TRIzol (ThermoFisher Scientific) RNA extraction procedure. The amount of RNA concentration was quantified, and RNA purity was assessed (based on absorbance values at 260nm/280nm and 260nm/230nm) using a spectrophotometer (NANODROP 2000 spectrophotometer, ThermoFisher Scientific, Mississauga, ON, Canada). Then each sample was diluted until the RNA concentration was ≤ 1300 ng/µl using nuclease-free water. An RNA concentration of 1000 ng/µl was used to synthesize cDNA using a High-Capacity Reverse Transcription kit (Applied Biosystems; Thermo Fisher Scientific, Foster City, CA). The calculated amount of nuclease-free water and RNA was added into each tube. The master mix was prepared using 10× RT Buffer, 25× dNTP mix (100nm), 10× RT random primers and MultiScribe reverse transcriptase, and then 5.8 µl of the master mix was added into each sample. The reaction was started at 25ºC and continued for 10 min, and then the temperature was raised to 37ºC for 2 h, and followed by 85ºC for 5 min. The program was run using a C1000 Touch Thermal Cycler (Bio-Rad Laboratories, Hercules, CA). The synthesized cDNA was stored at -20ºC until use for qPCR reactions. Primers used for the gene expression are shown in Table 4.2; some were designed using primer 3 in primerBLAST (NCBI). Each PCR reaction included 0.8 µl of 10 µM forward primer, 0.8 µl of 10 µM reverse primer, 6.4 µl of nuclease-free water and 10 µl of SsoFast^{TM} EvaGreen Supermix (Bio-Rad Laboratories, Inc., Hercules, CA). The PCR conditions for the primers were 1 × 95ºC for 30 s for initial denaturation, 40 cycles × (95ºC for 5 s for denaturation, annealing temperature for 5 s and 72ºC for 5 s for extension), followed by a melt curve analysis from 55ºC to 95ºC in 0.5ºC increments for 5 s each. The PCR of all the samples was run using a Bio-Rad CFX 96 Real-Time System (Bio-Rad Laboratories, Inc., Hercules, CA). The sequence and annealing temperature for each primer are mentioned in Table 4.2. The quantification of the products was done using a Bio-Rad CFX Manager Software, version 3.1 (Bio-Rad Laboratories, Inc., Hercules, CA). The mRNA abundance was determined based on a serial 5-fold dilution curve of a pooled cDNA of all the samples. Then the calculated mRNA abundance of genes of interest was normalized to the level of housekeeping genes. GAPDH and RPL30 were used as house-keeping genes to normalize the genes of interest in the ileum for the d 11 and 33, respectively. The average of GAPDH and RPL30 was used for the d 11, and RPL30 was used for the d 33 of normalizing the genes of interest in the caeca.

Table 4. 2. The primers used for qPCR

Target	Function	Orientation	Sequence (5'-3')	Size (bp)	Annealing temperature (ºC)	Reference
GAPDH	Glycolysis	f[1]	GTGAAAGTCGGAGTCAACGGA	101	60	Cheled-Shoval et al., 2011
		r[2]	AAGGGATCATTGATGGCCAC			
RPL30	Protein coding	f	GAGTCACCTGGGTCAATAA	160	57	Yang et al., 2013
		r	CCAACAACTGTCCTGCTTT			
IL-6	Pro-inflammatory cytokine	f	GAAATCCCTCCTCGCCAATCTGA	281	63	Bhanja et al., 2015
		r	TGAAACGGAACAACACTGCCATCT			
IL-8	Pro-inflammatory cytokine/ chemotaxis	f	ATGAACGGCAAGCTTGGAGCT	312	62	Khatri and Sharma, 2006
		r	TCACAGTGGTGCATCAGAATTGA			
MUC2	Mucus secretion	f	GCCTGCCCAGGAAATCAAG	59	55	Chen et al., 2015
		r	CGACAAGTTTGCTGGCACAT			
MUC5ac	Mucus secretion	f	TGTGGTTGCTATGAGAATGGA	244	60	Kitessa et al., 2014
		r	TTGCCATGGTTTGTGCAT			
AvBD2	Anti-microbial peptide	f	GGCGGGACATGCTGTTCT	107	60	Designed with Primer3 (NCBI)
		r	CCATTTGCAGCAGGAACG			
PCNA	DNA replication and repair	f	GGGTTCGGGCGGCATCAG	807	55	Withana Gamage MSc thesis
		r	TCTTCATTTCCAGCACACTTCAG			
MCT1	Mono-carboxylate transporter-1	f	CAAATCCATCACTGTGTTCTTCA	111	57	Designed with Primer3 (NCBI)
		r	GGACCTCCTGCATACATAACA			

[1] forward.
[2] reverse.

4.3.11 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. The treatments were organized according to a 3 × 3 factorial arrangement. Data were analyzed using a two-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, Carey, N.C. 2008). The significance level was $P \leq 0.05$. Mean separation was done using the Tukey-Kramer test. Data were tested for normality using the Shapiro-Wilk test, and log-transformed when they were not normally distributed.

4.4 Results

4.4.1. Ingredient nutrient composition

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 reported. Total β-glucan was analyzed as 8.70 and 0.64% in HB and wheat, respectively. The content of total starch, CP, fat, and ash were determined as 53.7, 16.2, 2.8 and 2.4%, respectively, in HB, and 62.8, 14.9, 1.2 and 1.7% in wheat.

4.4.2 Performance parameters

Dietary HB and BGase affected broiler performance in an age-dependent manner (Table 4.3). Except for BWG from 11 to 22 d and FI from 22 to 32 d, all production data were influenced by interactions between the main effects of HB and BGase. 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 the birds fed diets containing 0.01% BGase than the other two 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 two enzyme levels for the birds fed 60% HB. From 11 to 22 d, BWG was lower for the 60% HB birds in comparison to those from the 0 and 30% HB treatments: both levels of BGase

addition increased BWG for this time 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 two enzyme levels for the 60% HB diet. Feed to gain ratio was also not affected by enzyme level for the two 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 diets containing enzyme grew faster at 60% HB diets. With the same time 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. For F:G, the lowest value was achieved by the 0.1% compared to the 0.01% BGase level when no HB was fed. For the birds on the 30% HB diets, the 0.1% BGase treatment was superior to the un-supplemented 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 to 0 and 0.1% 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 compared to 0% BGase at both 0 and 30% HB diets, and 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 4.4). 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 destruction of the intestinal epithelial membrane and bacterial translocation as a result of subclinical coccidiosis. These data support the conclusion that vaccination with Coccivac-B52 induced a disease challenge in experimental birds.

Table 4. 3. Effects of hulless barley and β-glucanase on production performance of broiler chickens

HB[1] (%)	BGase (%)	BWG (kg)				FI (kg)				F:G			
		d 0-11	d 11-22	d 22-32	d 0-32	d 0-11	d 11-22	d 22-32	d 0-32	d 0-11	d 11-22	d 22-32	d 0-32
0	0	0.278^{ab}	0.641	0.960^{ab}	1.879^{abc}	0.338^{ab}	1.035^{a}	1.626	3.00^{a}	1.20^{ef}	1.49^{bc}	1.66^{cd}	1.53^{cde}
	0.01	0.275^{abc}	0.672	0.952^{ab}	1.899^{ab}	0.337^{ab}	1.037^{a}	1.633	3.006^{a}	1.20^{ef}	1.42^{c}	1.69^{c}	1.52^{def}
	0.1	0.286^{a}	0.668	0.977^{a}	1.931^{a}	0.343^{a}	1.037^{a}	1.581	2.962^{ab}	1.18^{f}	1.43^{c}	1.61^{d}	1.48^{f}
30	0	0.266^{bc}	0.641	0.919^{bc}	1.826^{c}	0.336^{ab}	1.038^{a}	1.591	2.965^{ab}	1.25^{cd}	1.50^{bc}	1.71^{c}	1.57^{c}
	0.01	0.275^{abc}	0.646	0.924^{bc}	1.846^{bc}	0.346^{a}	1.029^{a}	1.576	2.950^{ab}	1.22^{cde}	1.48^{c}	1.67^{cd}	1.53^{cde}
	0.1	0.277^{ab}	0.649	0.939^{ab}	1.865^{bc}	0.344^{a}	1.034^{a}	1.525	2.903^{bc}	1.21^{def}	1.48^{c}	1.60^{d}	1.49^{ef}
60	0	0.243^{d}	0.562	0.788^{e}	1.594^{e}	0.328^{b}	0.979^{b}	1.540	2.846^{cd}	1.32^{b}	1.62^{a}	1.94^{a}	1.72^{a}
	0.01	0.264^{c}	0.603	0.859^{d}	1.726^{d}	0.339^{ab}	1.033^{a}	1.569	2.941^{ab}	1.26^{c}	1.58^{ab}	1.80^{b}	1.63^{b}
	0.1	0.237^{d}	0.622	0.881^{cd}	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^{cd}
SEM[2]		0.0020	0.0047	0.0073	0.0122	0.0011	0.0039	0.0069	0.0100	0.0073	0.0095	0.0124	0.009
Main effects													
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
Probability													
HB		<.0001	<.0001	<.0001	<.0001	0.0002	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001
BGase		0.0004	0.0003	<.0001	<.0001	0.01	0.03	<.0001	<.0001	0.0004	0.0003	<.0001	<.0001
HB × BGase		<.0001	0.09	0.002	0.0002	0.04	0.0004	0.68	0.03	<.0001	0.02	<.0001	<.0001

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

[1]HB - hulless barley; BGase - β-glucanase; BWG - body weight gain; FI - feed intake; F:G - feed to gain ratio.

[2]SEM - pooled standard error of mean (n=9 pens per treatment).

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

Hulless barley (%)	β-glucanase (%)	Mortality (%)
	0	4.3
0	0.01	3.5
	0.1	4.0
	0	3.5
30	0.01	4.6
	0.1	4.5
	0	3.5
60	0.01	4.5
	0.1	3.1
SEM[1]		0.304
Main effects		
Hulless barley (%)		3.9
0		4.2
30		3.7
60		
β-glucanase (%)		3.7
0		4.2
0.01		3.9
0.1		
Probability		
Hulless barley		0.81
β-glucanase		0.82
Hulless barley × β-glucanase		0.72

[1]SEM - pooled standard error of mean (n=9 pens per treatment).

4.4.3 Beta-glucan molecular weight distribution

Interactions between HB and BGase were observed for all β-glucan MW parameters of the soluble ileal content of broiler chickens except for the Mw at d 33. Overall, Mp, Mw and MW-10% were lower in 0% HB treatments compared to 30 and 60% HB treatments. Further, molecular weight parameters were higher for the 60% HB compared to 30% HB level with 0% BGase supplementation at d 33.

In the birds fed 0% HB diets, MW-10% was lower with the addition of 0.1% BGase than with the 0 and 0.01% BGase levels, and Mw was lower with 0.1% BGase than with 0.01% BGase at d 11. When considering the birds fed 30% HB diets, both 0.01 and 0.1% BGase resulted in a lower Mp, Mw and MW-10% than with the 0% BGase level at d 11. In addition, MW-10% of β-glucan molecules was lower in the birds aged 11 d with 0.1% BGase supplementation than with 0.01% BGase level. For the birds fed 60% HB diets, both 0.01 and 0.1% BGase resulted in lower values for Mp, Mw and MW-10% compared to 0% BGase at d 11. Further, 0.1% BGase supplementation compared to 0.01% BGase level showed lower Mp and MW-10%. As an example, the blue lines at the same point ($1e^4$) of the horizontal axis in the graphs shown in Figures 4.1A and 4.1B demonstrate the β-glucan curve has shifted to the left side (smaller β-glucan molecules) with the 0.1% BGase compared to the 0% BGase when the birds were fed 60% HB-based diets. Moreover, a bimodal distribution of molecules was noticed when the diets were consisted of BGase (Figure 4.1B)

At d 33, MW-10% was lower with the addition of 0.1% BGase than with the 0 and 0.01% BGase levels in the birds fed 0% HB diets. For the 30% HB treatments, both Mp and MW-10% were lower with the addition of 0.01 and 0.1% BGase to the diets in comparison to the diets with 0% BGase. For the birds fed 60% HB diets, Mp and MW-10% decreased with the increasing level of BGase in the diets. Although the interaction was not found for Mw at d 33, Mw increased with increasing HB, while decreasing with increasing BGase in the diets.

Overall, MW parameters for 0% HB treatments were numerically similar at d 11 and 33, although d 33 values were appeared to be lower than d 11 in 30 and 60% HB treatments.

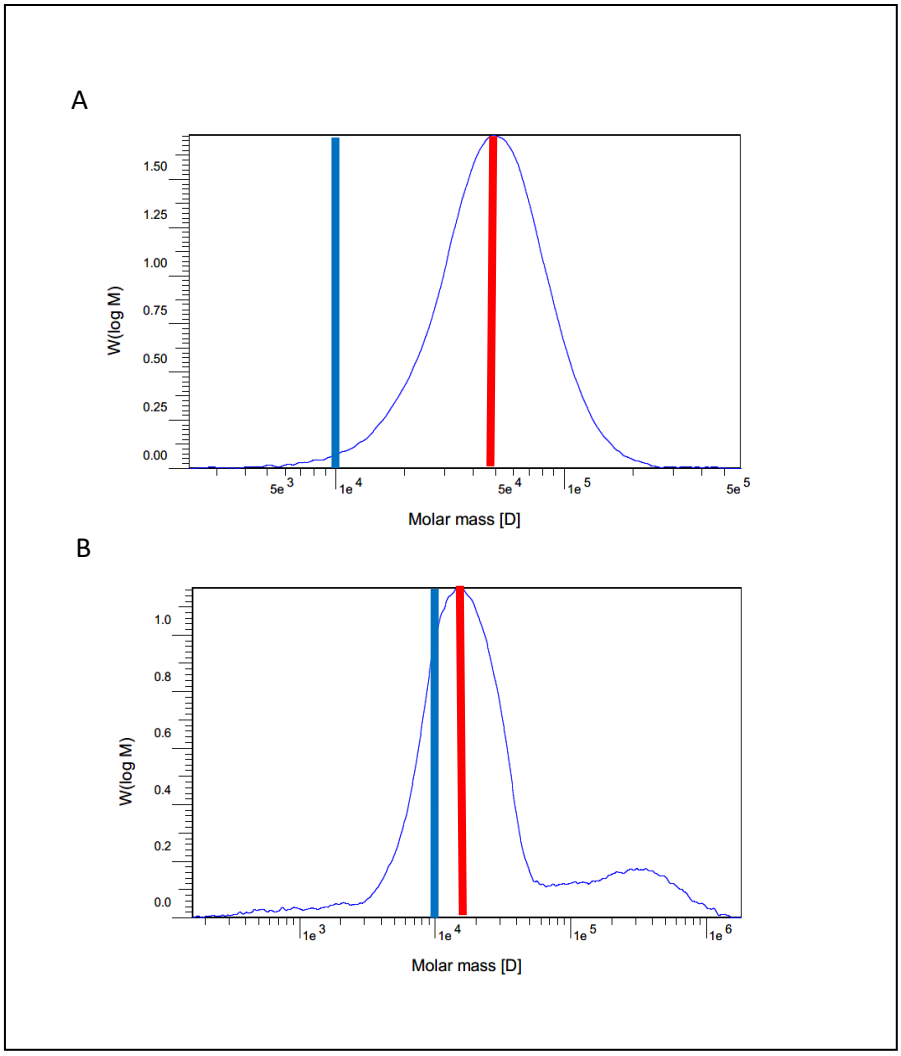

Figure 4. 1. Beta-glucan molecular weight distribution in soluble ileal digesta from broilers fed 60% hulless barley diets. Blue lines denote point 1e4 on the x-axis and red lines indicate the Mp of the distribution curve. A. 0% β-glucanase; B. 0.1% β-glucanase

Table 4. 5. Effects of hulless barley and β-glucanase on β-glucan molecular weight in the soluble ileal content of broiler chickens

HB[1] (%)	β-glucanase (%)	Molecular weight (g/mol)					
		d 11			d 33		
		Mp[2]	Mw	MW-10%	Mp	Mw	MW-10%
0	0	37056^{bc}	42779de	20325^{b}	36633^{c}	42391	21061^{b}
	0.01	45834^{b}	47864^{cd}	18623^{b}	33697^{cd}	37326	17855^{b}
	0.1	29534^{bc}	28659^{e}	10691^{cd}	26386^{cde}	29852	10039^{cd}
30	0	80837^{a}	80759^{ab}	37329^{a}	53072^{b}	57846	20471^{b}
	0.01	45341^{b}	50488^{cd}	24771^{b}	32500^{cd}	44226	12457^{c}
	0.1	27570^{bc}	48635^{cd}	8251^{d}	23664de	37806	8719^{cd}
60	0	78293^{a}	80971^{a}	33322^{a}	71377^{a}	71684	28973^{a}
	0.01	42727^{b}	50008^{cd}	17430^{bc}	33677^{cd}	58350	11734^{c}
	0.1	23611^{c}	62930^{bc}	7632^{d}	16985^{e}	48316	7074^{d}
SEM[3]		3050.8	2535.9	1490.8	2298.7	1926.3	987.1
Main effects							
HB (%)							
0		37475	39767	16546	32238	36523^{c}	16319
30		51249	59961	23450	36412	46626^{b}	13822
60		48210	64636	19461	40680	59450^{a}	15927
β-glucanase (%)							
0		65395	68170	30325	53694	57307^{a}	23502
0.01		44634	49453	20275	33291	46634^{b}	14016
0.1		26905	46741	8858	22345	38658^{c}	8610
Probability							
HB		0.0006	<.0001	<.0001	0.001	<.0001	0.002
β-glucanase		<.0001	<.0001	<.0001	<.0001	<.0001	<.0001
HB × β-glucanase		<.0001	<.0001	<.0001	<.0001	0.39	<.0001

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

[1]HB - hulless barley

[2]Mp - peak MW; Mw - weight average MW; MW-10% - The maximum MW for the smallest 10% β-glucan molecules.

[3]SEM - pooled standard error of mean (n=6 birds per treatment).

4.4.4 Viscosity

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

Table 4. 6. Effects of hulless barley and β-glucanase on ileal digesta viscosity of broiler chickens

Hulless barley (%)	β-glucanase (%)	Viscosity (cP)	
		d 11	d 33
0	0	7.84	2.37[b]
	0.01	3.48	2.36[b]
	0.1	3.37	2.83[ab]
30	0	7.11	3.67[ab]
	0.01	6.93	3.38[ab]
	0.1	3.66	3.13[ab]
60	0	9.73	3.98[a]
	0.01	5.31	3.46[ab]
	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
Probability			
Hulless barley		0.26	0.002
β-glucanase		<.0001	0.09
Hulless barley × β-glucanase		0.16	0.02

[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 (d 11; n=6 birds per treatment/d 33; n=9 birds per treatment).

4.4.5 Short chain fatty acids and gastro-intestinal pH

Interactions of main effects of HB and BGase, or the main effects did not affect the ileal total SCFA or major individual SCFA concentrations (acetic acid, propionic acid, butyric acid, lactic acid) at 11 d old broiler chickens (Table 4.7). However, interactions between HB and BGase were found for the valeric, isovaleric and caproic acid concentrations at d 11. Although these interactions were significant, no clear trends were identified in relationship to grain source or enzyme level. When examined based on the molar percentages of SCFA, interactions between main effects were noted for all categories. However, variation in values was minor and clear trends were not identified.

Interactions between the main effects were found for the concentrations of total SCFA and all individual SCFA concentrations in the caeca of broiler chickens aged 11 d (Table 4.8). Differences were small, and the most notable effect was the increase in SCFA values for the 60% HB combined with 0.1% BGase treatment. Interactions were found for the molar percentages of butyric and valeric acids, but the trends were not clear.

Dietary treatment did not affect the concentrations of total or major SCFA in the ileum of 33 d broilers (Table 4.9). However, BGase tended to increase total SCFA ($P = 0.06$), acetic acid ($P = 0.10$), propionic acid ($P = 0.06$) and lactic acid ($P = 0.08$). Interactions between HB and BGase were significant for the ileal concentrations of isobutyric, valeric, isovaleric and caproic acids. Except for isobutyric acid, all the other minor SCFA concentrations were higher with increasing BGase, when the birds were fed 30 and 60% HB diets. Interactions were noted for the molar percentages of caecal butyric, isobutyric, valeric, isovaleric and caproic acids, but were consisted of minor trends. The percentage of acetic acid was lower with the increasing level of BGase. Further, lactic acid percentage was higher with increasing HB, whereas lower with increasing BGase in the diets.

The HB and BGase interactions were not significant for caecal SCFA concentrations, but the use of an enzyme impacted caecal SCFA levels at 33 d of age (Table 4.10). Total, butyric acid, valeric acid and isovaleric acid values were lower for the 0.01% compared to the 0% BGase treatment, and either numerically or statistically lower than the 0.1% enzyme level. Acetic acid

(P = 0.06), propionic acid (P = 0.06) and caproic acid (P = 0.07) levels also tended to be lowest for 0.01% BGase treatment. Interactions were found between HB and BGase levels for the molar proportion of acetic, propionic and caproic acids in caecal digesta, but the differences were small. Enzyme level affected isovaleric acid with the 0.01% BGase level resulting in lower values than either the 0 or 0.1% BGase treatments.

Hulless barley and BGase affected the pH of GI contents at both d 11 and 33 (Table 4.11). There was an interaction between HB and BGase levels for the crop pH at d 11. Statistical separation of interaction means demonstrated that the pH of birds fed the 0% HB diet with 0.01% BGase was higher than those fed the wheat diet without enzyme, and the two enzyme levels in the 60% HB diet; all other values were intermediate, and not different than the extremes. Gizzard, duodenum and jejunum pH values were not affected by dietary treatment. Ileal pH increased with levels of HB and BGase. The interaction between HB and BGase was significant for caecal pH. The highest level of BGase compared to 0% BGase significantly decreased the caecal pH in the birds fed 60% HB diets.

At d 33, crop and gizzard pH values were not affected by treatment. An interaction between main effects was found for duodenal pH, where the highest level of BGase increased duodenal pH compared to 0% BGase when given a wheat-based diet. Jejunal pH increased with an increasing level of BGase, while both HB and BGase increased ileal pH. Caecal pH was higher at 30% HB compared to 0 and 60% HB in the diets.

Table 4. 7. Effects of hulless barley and β-glucanase on ileal short chain fatty acids of broiler chickens aged 11 days

HB[1] (%)	BGase (%)	SCFA µmol/g of wet ileal content								Molar percentage of total SCFA						
		Total	Ace	Pro	Buty	Val	Isov	Cap	Lac	Ace	Pro	Buty	Val	Isov	Cap	Lac
0	0	126.1	48.0	18.5	8.2	2.4^{a}	2.7^{a}	1.1^{a}	44.9	38.0^{ab}	14.7^{ab}	6.5^{b}	1.9^{abc}	2.1^{a}	0.9^{ab}	35.6^{b}
	0.01	117.0	45.6	17.2	7.7	0.8^{b}	2.5^{abc}	1.1^{a}	42.0	38.8^{ab}	14.7^{ab}	6.6^{ab}	0.6^{d}	2.1^{a}	1.0^{a}	35.9^{ab}
	0.1	118.0	44.9	16.8	7.8	2.5^{a}	2.6^{ab}	1.1^{a}	42.0	38.0^{ab}	14.3^{b}	6.5^{ab}	2.1^{ab}	2.1^{a}	1.0^{a}	35.6^{b}
30	0	119.6	46.8	17.8	8.0	1.1^{b}	0.8^{c}	1.1^{a}	43.6	39.2^{a}	14.9^{a}	6.7^{ab}	0.9^{cd}	0.7^{b}	1.0^{a}	36.4^{ab}
	0.01	122.4	46.7	18.4	8.3	0.9^{b}	0.9^{c}	0.4^{b}	46.4	38.3^{ab}	15.0^{a}	6.8^{a}	0.8^{cd}	0.8^{b}	0.3^{c}	37.6^{a}
	0.1	120.4	45.7	17.5	7.8	2.5^{a}	2.6^{ab}	1.1^{a}	42.9	37.9^{ab}	14.5^{ab}	6.5^{b}	2.1^{ab}	2.1^{a}	0.9^{ab}	35.6^{b}
60	0	125.3	48.2	18.4	8.2	2.7^{a}	1.5^{abc}	1.1^{a}	44.9	38.4^{ab}	14.6^{ab}	6.6^{ab}	2.1^{ab}	1.2^{ab}	1.0^{a}	35.8^{ab}
	0.01	122.4	45.3	17.8	8.1	2.7^{a}	2.7^{a}	1.2^{a}	43.7	37.4^{b}	14.6^{ab}	6.6^{ab}	2.2^{a}	2.2^{a}	1.0^{a}	35.8^{ab}
	0.1	122.5	47.6	18.3	8.1	1.5^{ab}	1.4^{bc}	0.7^{ab}	44.6	38.8^{ab}	14.9^{a}	6.6^{ab}	1.2^{bcd}	1.1^{b}	0.6^{bc}	36.4^{ab}
SEM[2]		1.40	0.49	0.20	0.10	0.11	0.12	0.04	0.60	0.11	0.04	0.02	0.09	0.09	0.03	0.14
Main effects																
HB (%)																
0		120.4	46.2	17.5	7.9	1.9	2.6	1.1	43.0	38.3	14.6	6.5	1.6	2.1	0.9	35.7
30		120.8	46.4	17.9	8.0	1.5	1.5	0.9	44.3	38.5	14.8	6.6	1.3	1.2	0.7	36.5
60		123.2	47.0	18.1	8.1	2.3	1.9	1.0	44.4	38.2	14.7	6.6	1.8	1.5	0.8	36.0
BGase (%)																
0		123.7	47.7	18.2	8.1	2.1	1.7	1.1	44.5	38.5	14.7	6.5	1.6	1.3	0.9	35.9
0.01		120.4	45.9	17.8	8.0	1.5	2.0	0.9	44.0	38.2	14.8	6.7	1.2	1.7	0.7	36.4
0.1		120.3	46.0	17.5	7.9	2.2	2.2	1.0	43.2	38.2	14.6	6.6	1.8	1.8	0.8	35.9
Probability																
HB		0.69	0.77	0.53	0.68	0.003	0.0001	0.03	0.68	0.65	0.08	0.18	0.004	0.0004	0.02	0.07
BGase		0.53	0.24	0.31	0.72	0.02	0.10	0.01	0.65	0.36	0.20	0.20	0.01	0.04	0.02	0.37
HB × BGase		0.72	0.64	0.39	0.80	<.0001	<.0001	<.0001	0.55	0.01	0.005	0.03	<.0001	<.0001	<.0001	0.02

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

[1]HB - hulless barley; BGase - β-glucanase; SCFA - short chain fatty acids; Ace - Acetic acid; Pro - Propionic acid; Buty - Butyric acid; Isob - Isobutyric acid; Val - Valeric acid; Isov - Isovaleric acid; Cap - Caproic acid; Lac - Lactic acid.

[2]SEM - pooled standard error of mean (n=12 birds per treatment).

Table 4. 8. Effects of hulless barley and β-glucanase on caecal short chain fatty acids of broiler chickens aged 11 days

HB[1]	BGase	SCFA µmol/g of wet caecal content								Molar percentage of total SCFA						
(%)	(%)	Total	Ace	Pro	Buty	Isob	Val	Isov	Cap	Ace	Pro	Buty	Isob	Val	Isov	Cap
	0	266.0^{ab}	157.1^{ab}	58.0^{ab}	26.4^{ab}	8.6^{ab}	3.4^{c}	8.61^{ab}	3.7^{ab}	58.9	21.7	9.96^{a}	3.2	1.3^{b}	3.2	1.3
0	0.01	245.2^{ab}	143.1^{ab}	52.1^{ab}	24.1^{ab}	7.8^{ab}	7.7^{ab}	6.87^{ab}	3.3^{ab}	58.3	21.2	9.85^{ab}	3.1	3.1^{a}	2.8	1.3
	0.1	217.9^{b}	126.8^{b}	46.2^{b}	21.3^{b}	6.9^{b}	6.7^{abc}	6.85^{b}	2.9^{b}	58.0	21.2	9.80^{ab}	3.1	3.1^{a}	3.1	1.3
	0	285.0^{ab}	166.6^{ab}	60.2^{ab}	27.4^{ab}	9.0^{ab}	8.8^{a}	8.97^{ab}	3.8^{ab}	58.3	21.1	9.65^{b}	3.1	3.1^{a}	3.1	1.3
30	0.01	241.7^{ab}	140.0^{ab}	51.4^{ab}	24.0^{ab}	7.7^{ab}	7.6^{ab}	7.66^{ab}	3.2^{ab}	57.9	21.2	9.90^{ab}	3.1	3.1^{a}	3.1	1.3
	0.1	223.4^{ab}	131.9^{ab}	49.2^{ab}	22.3^{ab}	7.4^{ab}	7.3^{abc}	7.34^{ab}	3.1^{ab}	57.6	21.5	9.78^{ab}	3.2	3.1^{a}	3.2	1.3
	0	224.6^{ab}	131.8^{ab}	48.9^{ab}	22.3^{ab}	7.3^{ab}	4.2^{bc}	7.31^{ab}	2.6^{b}	58.6	21.8	9.95^{a}	3.2	1.7^{b}	3.2	1.2
60	0.01	208.2^{b}	121.0^{b}	44.2^{b}	20.3^{b}	6.6^{b}	6.4^{abc}	6.58^{b}	2.8^{b}	58.1	21.2	9.78^{ab}	3.1	3.1^{a}	3.1	1.3
	0.1	309.4^{a}	178.1^{a}	66.9^{a}	30.2^{a}	10.0^{a}	9.9^{a}	9.92^{a}	4.2^{a}	57.5	21.6	9.78^{ab}	3.2	3.1^{a}	3.2	1.3
SEM[2]		7.46	4.32	1.58	0.71	0.23	0.35	0.25	0.10	0.09	0.05	0.01	0.01	0.11	0.04	0.01
Main effects																
HB (%)																
0		243.0	142.3	52.1	24.0	7.8	5.9	7.44	3.3	58.6	21.4	9.8	3.2	2.5	3.0	1.3
30		250.0	146.2	53.6	24.6	8.0	7.9	7.99	3.4	58.1	21.3	9.7	3.2	3.1	3.1	1.3
60		247.4	143.6	53.3	24.3	8.0	6.8	7.93	3.2	57.7	21.5	9.8	3.2	2.6	3.2	1.3
BGase (%)																
0		258.5	151.8	55.7	25.4	8.3	5.5	8.29	3.4	58.4^{a}	21.6	9.8	3.2	2.0	3.2	1.3
0.01		231.7	134.7	49.2	22.8	7.3	7.2	7.03	3.1	57.9^{b}	21.2	9.8	3.1	3.1	3.0	1.3
0.1		250.2	145.6	54.1	24.6	8.1	7.9	8.04	3.4	58.1^{b}	21.4	9.7	3.2	3.1	3.1	1.3
Probability																
HB		0.91	0.92	0.90	0.93	0.87	0.03	0.58	0.81	0.06	0.26	0.10	0.41	0.02	0.32	0.59
BGase		0.28	0.23	0.18	0.29	0.19	0.003	0.08	0.39	0.001	0.06	0.27	0.12	<.0001	0.22	0.65
HB × BGase		0.001	0.002	0.001	0.002	0.001	0.001	0.01	0.001	0.91	0.12	0.004	0.17	0.02	0.43	0.50

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

[1]HB - hulless barley; BGase - β-glucanase; SCFA - short chain fatty acids; Ace - Acetic acid; Pro - Propionic acid; Buty - Butyric acid; Isob - Isobutyric acid; Val - Valeric acid; Isov - Isovaleric acid; Cap - Caproic acid.; Lac - Lactic acid.

[2]SEM - pooled standard error of mean (n=12 birds per treatment).

Table 4. 9. Effects of hulless barley and β-glucanase on ileal short chain fatty acids of broiler chickens aged 33 days

HB[1] (%)	BGase (%)	SCFA µmol/g of wet ileal content									Molar percentage of total SCFA							
		Total	Ace	Pro	Buty	Isob	Val	Isov	Cap	Lac	Ace	Pro	Buty	Isob	Val	Isov	Cap	Lac
0	0	121.4	46.6	17.6	7.9	0.14^{b}	2.6^{a}	2.4^{abc}	1.1^{a}	42.8	38.4	14.5	6.53^{bcd}	0.12^{b}	2.1^{a}	2.0^{ab}	0.9^{a}	35.33
	0.01	119.3	45.6	17.3	7.7	0.00^{b}	2.5^{a}	2.5^{ab}	1.1^{a}	42.2	38.2	14.5	6.51^{cd}	0.00^{b}	2.1^{a}	2.1^{a}	0.9^{a}	35.43
	0.1	124.5	47.7	18.1	8.1	0.00^{b}	2.6^{a}	2.5^{ab}	1.1^{a}	44.2	38.3	14.5	6.50^{cd}	0.00^{b}	2.1^{a}	2.0^{ab}	0.9^{a}	35.48
30	0	117.2	45.4	16.5	7.7	1.54^{a}	1.4^{b}	1.7^{bc}	0.7^{bc}	41.9	38.8	14.0	6.65^{ab}	1.34^{a}	1.1^{b}	1.4^{b}	0.6^{b}	35.87
	0.01	125.6	47.8	18.1	8.1	0.00^{b}	2.6^{a}	2.7^{a}	1.1^{a}	44.8	38.1	14.4	6.48^{d}	0.00^{b}	2.1^{a}	2.1^{a}	0.9^{a}	35.67
	0.1	120.7	46.3	17.4	7.8	0.14^{b}	2.5^{a}	2.5^{ab}	1.1^{a}	42.6	38.3	14.4	6.49^{cd}	0.12^{b}	2.1^{a}	2.1^{a}	0.9^{a}	35.32
60	0	115.2	44.6	17.0	7.6	0.00^{b}	1.5^{b}	1.6^{c}	1.0^{ab}	41.6	38.7	14.8	6.62^{abc}	0.00^{b}	1.3^{b}	1.4^{b}	0.8^{a}	36.11
	0.01	123.7	48.0	18.2	8.2	0.17^{b}	1.6^{b}	1.9^{abc}	0.6^{c}	44.6	38.8	14.7	6.67^{a}	0.11^{b}	1.3^{b}	1.5^{ab}	0.5^{b}	36.11
	0.1	125.0	47.8	18.1	8.1	0.00^{b}	2.6^{a}	2.7^{a}	1.1^{a}	44.3	38.2	14.5	6.50^{cd}	0.00^{b}	2.1^{a}	2.1^{a}	0.9^{a}	35.44
SEM[2]		1.01	0.37	0.16	0.06	0.05	0.07	0.07	0.02	0.35	0.05	0.06	0.01	0.05	0.05	0.05	0.02	0.06
Main effects																		
HB (%)																		
0		121.7	46.6	17.6	7.9	0.04	2.6	2.5	1.1	43.1	38.3	14.5	6.51	0.04	2.1	2.0	0.9	35.41^{b}
30		121.2	46.5	17.4	7.9	0.56	2.2	2.3	1.0	43.1	38.4	14.3	6.54	0.49	1.8	1.9	0.8	35.62^{ab}
60		121.3	46.8	17.8	8.0	0.05	1.9	2.1	0.9	43.5	38.6	14.6	6.60	0.03	1.6	1.7	0.7	35.88^{a}
BGase (%)																		
0		117.9	45.5	17.0	7.7	0.56	1.8	1.9	0.9	42.1	38.6^{a}	14.4	6.60	0.48	1.5	1.6	0.8	35.77^{a}
0.01		122.9	47.2	17.9	8.0	0.05	2.3	2.4	0.9	43.9	38.4^{ab}	14.5	6.55	0.03	1.8	1.9	0.8	35.74^{ab}
0.1		123.4	47.3	17.9	8.0	0.04	2.6	2.6	1.1	43.7	38.3^{b}	14.5	6.50	0.04	2.1	2.1	0.9	35.41^{b}
Probability																		
HB		0.97	0.92	0.60	0.83	<.001	0.001	0.02	0.009	0.87	0.08	0.08	0.002	<.0001	0.0001	0.01	0.01	0.01
BGase		0.06	0.10	0.06	0.18	<.001	<.001	0.001	0.003	0.08	0.03	0.69	0.0002	<.0001	<.0001	0.0004	0.01	0.03
HB × BGase		0.24	0.26	0.39	0.25	<.001	0.001	0.02	<.001	0.25	0.06	0.47	0.001	<.0001	0.0001	0.01	<.0001	0.13

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

[1]HB - hulless barley; BGase - β-glucanase; SCFA - short chain fatty acids; Ace - Acetic acid; Pro - Propionic acid; Buty - Butyric acid; Isob - Isobutyric acid; Val - Valeric acid; Isov - Isovaleric acid; Cap - Caproic acid.; Lac - Lactic acid.

[2]SEM - pooled standard error of mean (n=18 birds per treatment).

Table 4. 10. Effects of hulless barley and β-glucanase on caecal short chain fatty acids of broiler chickens aged 33 days

HB[1] (%)	BGase (%)	SCFA µmol/g of wet caecal content								Molar percentage of total SCFA						
		Total	Ace	Pro	Buty	Isob	Val	Isov	Cap	Ace	Pro	Buty	Isob	Val	Isov	Cap
	0	229.4	132.5	49.1	22.9	7.3	7.1	7.2	3.0	57.6^{b}	21.4^{ab}	10.0	3.1	3.1	3.1	1.35^{ab}
0	0.01	203.6	120.0	42.9	20.0	6.4	6.3	5.1	2.7	58.9^{a}	21.0^{abc}	9.8	3.1	3.1	2.5	1.33^{abc}
	0.1	197.3	114.8	41.6	19.6	6.2	6.1	6.1	2.6	58.1^{ab}	21.1^{abc}	9.9	3.1	3.1	3.1	1.34^{abc}
	0	225.6	131.6	47.3	22.5	7.0	6.9	7.0	3.0	58.3^{ab}	21.0^{abc}	10.0	3.1	3.0	3.1	1.33^{abc}
30	0.01	200.3	116.4	42.8	20.0	6.4	4.2	3.6	2.7	59.1^{ab}	21.5^{a}	10.0	3.2	3.1	2.5	1.37^{a}
	0.1	241.2	141.4	50.4	24.2	7.0	7.4	7.4	3.2	58.6^{ab}	21.0^{abc}	10.0	2.9	3.0	3.0	1.33^{abc}
	0	224.5	131.9	46.4	22.5	6.9	6.8	6.8	2.9	58.8^{a}	20.6^{c}	10.0	3.0	3.0	3.0	1.30^{c}
60	0.01	212.4	124.3	44.2	21.7	6.6	6.1	6.5	2.8	58.5^{ab}	20.8^{c}	10.2	3.1	2.8	3.0	1.32^{bc}
	0.1	231.1	135.2	48.3	23.0	7.2	7.1	7.1	3.0	58.5^{ab}	20.9^{bc}	9.9	3.1	3.0	3.0	1.32^{bc}
SEM[2]		3.63	2.13	0.77	0.35	0.12	0.15	0.12	0.04	0.08	0.05	0.02	0.02	0.02	0.04	0.003
Main effects																
HB (%)																
0		210.1	122.4	44.5	20.9	6.6	6.5	6.1	2.8	58.2	21.2	9.9	3.1	3.1	2.9	1.34
30		222.3	129.8	46.8	22.2	6.8	6.9	6.5	2.9	58.3	21.1	10.0	3.1	3.1	2.9	1.34
60		222.7	130.5	46.3	22.4	6.9	6.6	6.8	2.9	58.6	20.7	10.0	3.1	3.0	3.0	1.31
BGase (%)																
0		226.5^{a}	132.0	47.6	22.7^{a}	7.1	7.0^{a}	7.0^{a}	3.0	58.2	21.0	10.0	3.1	3.0	3.1^{a}	1.33
0.01		205.3^{b}	120.2	43.3	20.6^{b}	6.4	6.2^{b}	5.6^{b}	2.7	58.5	21.1	10.0	3.1	3.0	2.7^{b}	1.34
0.1		223.2^{ab}	130.5	46.7	22.3^{ab}	6.8	6.9^{ab}	6.9^{a}	2.9	58.4	20.9	10.0	3.0	3.0	3.1^{a}	1.33
Probability																
HB		0.25	0.21	0.43	0.13	0.63	0.48	0.15	0.35	0.10	0.0002	0.12	0.39	0.06	0.27	0.0003
BGase		0.04	0.06	0.06	0.04	0.13	0.03	0.0001	0.07	0.32	0.30	0.84	0.22	0.78	0.0006	0.23
HB × BGase		0.12	0.11	0.11	0.08	0.38	0.17	0.07	0.13	0.004	0.007	0.09	0.13	0.32	0.06	0.03

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

[1]HB - hulless barley; BGase - β-glucanase; SCFA - short chain fatty acids; Ace - Acetic acid; Pro - Propionic acid; Buty - Butyric acid; Isob - Isobutyric acid; Val - Valeric acid; Isov - Isovaleric acid; Cap - Caproic acid.; Lac - Lactic acid.

[2]SEM - pooled standard error of mean (n=18 birds per treatment).

Table 4. 11. Effects of hulless barley and β-glucanase on gastro-intestinal pH of broiler chickens

HB[1] (%)	BGase (%)	pH											
		d 11						d 33					
		Crop	Gizzard	Duodenum	Jejunum	Ileum	Caeca	Crop	Gizzard	Duodenum	Jejunum	Ileum	Caeca
0	0	4.58^{b}	2.68	6.00	5.87	6.26	6.03^{abc}	4.93	3.35	5.78^{b}	5.92	6.51	6.38
	0.01	5.19^{a}	2.63	6.00	5.92	6.37	6.24^{ab}	4.96	3.72	5.99^{ab}	5.95	6.55	6.14
	0.1	4.80^{ab}	2.69	6.00	5.96	6.23	5.90^{bc}	5.03	3.38	6.07^{a}	6.05	6.67	6.22
30	0	4.76^{ab}	2.45	5.96	5.86	6.17	6.37^{a}	4.85	3.56	6.09^{a}	5.99	6.44	6.61
	0.01	4.82^{ab}	2.73	6.08	5.98	6.25	5.91^{bc}	4.81	3.47	6.12^{a}	5.97	6.57	6.46
	0.1	4.78^{ab}	2.58	6.02	5.91	6.56	6.06^{abc}	4.99	3.43	6.24^{a}	6.04	6.79	6.45
60	0	4.78^{ab}	2.81	5.88	5.91	6.29	6.36^{a}	4.94	3.67	6.15^{a}	5.93	6.50	6.22
	0.01	4.69^{b}	2.48	5.93	5.88	6.54	6.17^{abc}	5.03	3.44	6.21^{a}	6.00	7.01	6.43
	0.1	4.62^{b}	2.41	5.99	5.92	6.61	5.78^{c}	4.83	3.44	6.01^{ab}	5.99	6.94	6.03
SEM[2]		0.034	0.034	0.018	0.014	0.035	0.035	0.033	0.036	0.023	0.012	0.043	0.036
Main effects													
HB (%)													
0		4.86	2.67	6.00	5.92	6.29^{b}	6.06	4.97	3.48	5.95	5.97	6.58^{b}	6.25^{b}
30		4.78	2.59	6.02	5.91	6.33^{ab}	6.11	4.88	3.48	6.15	5.99	6.60^{ab}	6.51^{a}
60		4.70	2.57	5.93	5.90	6.48^{a}	6.10	4.93	3.51	6.12	5.97	6.82^{a}	6.23^{b}
BG (%)													
0		4.71	2.64	5.95	5.88	6.24^{b}	6.25	4.91	3.53	6.01	5.95^{b}	6.48^{b}	6.41
0.01		4.90	2.61	6.00	5.93	6.39^{ab}	6.11	4.93	3.54	6.10	5.97^{ab}	6.71^{a}	6.34
0.1		4.73	2.56	6.01	5.93	6.47^{a}	5.91	4.95	3.41	6.10	6.03^{a}	6.80^{a}	6.23
Probability													
HB		0.13	0.42	0.13	0.90	0.03	0.75	0.51	0.92	<.0001	0.53	0.02	0.001
BGase		0.04	0.61	0.32	0.27	0.01	0.0001	0.85	0.28	0.09	0.02	0.004	0.10
HB × BGase		0.01	0.20	0.66	0.28	0.08	0.002	0.40	0.07	0.004	0.43	0.30	0.10

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

[1]HB - hulless barley; BG - β-glucanase.

[2]SEM - pooled standard error of mean (n=18 birds per treatment).

4.4.6 Gastro-intestinal wall histomorphology and gene expression

There were only minor differences of GI histological measurements of broiler chickens at both d 11 and 33, and no interactions were found (Table 4.12). At d 11, villi width was lower for the birds fed 0.01% BGase in comparison to the broilers from the 0% BGase treatment. However, no differences were found for the birds fed the highest level of BGase. The number of goblet cells (neutral) per villi tended to increase with the level of HB (P = 0.06) at d 11. At d 33, villi height decreased with an increasing level of BGase addition. The highest level of BGase increased the crypt depth compare to 0% BGase diets.

Both HB and BGase affected ileal gene expression in broiler chickens (Table 4.13), although statistical differences were minor. The RNA expression of IL6 in the ileum increased with the highest level of BGase compared to without enzyme treatments at d 11. Further, IL6 expression was higher at the 30% HB compared to the 0% HB levels. There was no treatment effect on ileal IL8, MUC2 and PCNA expression at d 11. An interaction was found for MCT1 expression at d 11. The 0% BGase resulted in the highest, while 0.01% BGase showed the lowest MCT1 expression, and all the other treatment means were intermediate and equal according to mean separation. The interaction between HB and BGase was significant for the ileal AvBD2 expression at d 11. Beta-glucanase dosages of 0.01 and 0.1% at the 30% HB level had the highest, whereas 0% BGase level at the 30% HB had the lowest AvBD2 expression, and all the other treatments showed intermediate and statistically similar means.

No interactions between HB and BGase were found for ileal gene expression at d 33, however there were significant main effects. The expression of IL8 was higher with increasing level of BGase in the diets, whereas MUC2 expression was lower with increasing BGase supplementation. In addition, MCT1 expression was lower at 0.01 and 0.1% BGase compared to 0% BGase treatments.

At d 11, no interaction was noted for caecal MUC2 expression, however it was higher at 60 compared to the 30% HB. Further, MUC2 expression was higher for the 0.1 than the 0% BGase level. Interactions were observed for caecal MUC5ac and MCT1 expression. The expression of MUC5ac was higher at the 0 compared to the 0.01% BGase level when the birds

were fed 0% HB diets, however no significant differences were noted due to enzyme level at the 30 and 60% HB levels. There were very minor differences in MCT1 expression, even though an interaction was found (Table 4.14). No treatment effects were found for caecal PCNA expression.

There were no treatment effects on caecal gene expression in 33 d old broiler chickens.

Table 4. 12. Effects of hulless barley and β-glucanase on histomorphology parameters in the ileum of broiler chickens

HB[1] (%)	β-glucanase (%)	d 11							d 33						
		Villi height (μm)	Villi width (μm)	Number of goblet cells/villus			Crypt depth (μm)	Villi height: Crypt depth	Villi height (μm)	Villi width (μm)	Number of goblet cells/villus			Crypt depth (μm)	Villi height: Crypt depth
				Acidic	Neutral	Mixed					Acidic	Neutral	Mixed		
	0	479	102	41	10	5	130	3.7	709	113	78	19	8	138	5.2
0	0.01	490	101	45	14	8	128	4.2	710	130	79	23	12	140	5.2
	0.1	461	103	39	10	4	115	4.2	725	113	67	15	6	160	4.6
	0	402	106	43	13	6	124	3.2	625	113	75	22	10	144	4.8
30	0.01	441	89	39	11	5	120	3.7	703	119	75	19	8	159	4.4
	0.1	465	100	39	13	7	136	3.6	776	117	72	21	9	161	4.9
	0	403	102	30	13	5	136	3.1	662	116	78	20	8	134	5.0
60	0.01	440	91	41	18	10	126	3.6	608	121	74	24	11	132	4.6
	0.1	446	93	35	17	6	139	3.2	652	117	64	21	10	161	4.1
SEM[2]		17.0	1.5	2.0	0.8	0.6	3.9	0.2	13.6	1.7	2.0	1.3	0.6	3.4	0.1
Main effects															
HB (%)															
0		477	102	42	11	6	125	4.0	715[a]	118	75	19	9	146	5.0
30		436	98	41	12	6	126	3.5	701[ab]	116	74	21	9	155	4.7
60		430	95	35	16	7	134	3.3	641[b]	118	72	22	9	142	4.6
β-glucanase (%)															
0		428	103[a]	38	12	5	130	3.3	665	114	77	20	9	139[b]	5.0
0.01		457	94[b]	42	14	8	125	3.8	674	123	76	22	10	144[ab]	4.7
0.1		458	99[ab]	38	13	6	130	3.7	718	115	68	19	8	161[a]	4.5
Probability															
HB		0.53	0.16	0.35	0.06	0.64	0.56	0.11	0.04	0.82	0.85	0.75	0.93	0.31	0.22
β-glucanase		0.74	0.03	0.71	0.58	0.21	0.81	0.31	0.21	0.06	0.15	0.62	0.47	0.03	0.16
HB × β-glucanase		0.96	0.41	0.77	0.42	0.42	0.76	0.98	0.28	0.61	0.93	0.73	0.28	0.74	0.32

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

[1]HB - hulless barley.

[2]SEM - pooled standard error of mean (n=6 birds per treatment).

Table 4. 13. Effects of hulless barley and β-glucanase on relative gene expression in the ileum of broiler chickens

HB[1] (%)	β-glucanase (%)	d 11						d 33					
		IL-6	IL-8	MUC2	PCNA	MCT1	AvBD2	IL-6	IL-8	MUC2	PCNA	MCT1	AvBD2
	0	0.53	0.75	0.81	1.22	1.19^{a}	0.80^{ab}	0.59	0.37	1.09	0.98	0.71	0.17
0	0.01	0.44	0.60	0.88	1.00	0.77^{b}	0.60^{ab}	0.59	0.62	0.94	1.04	0.70	0.91
	0.1	0.61	0.67	1.03	1.28	1.03^{ab}	0.80^{ab}	0.95	0.50	0.63	0.78	0.68	0.49
	0	0.38	0.58	0.88	0.94	0.79^{ab}	0.22^{b}	0.45	0.28	0.99	0.81	0.59	0.76
30	0.01	1.73	0.58	0.88	1.18	1.04^{ab}	2.21^{a}	0.49	0.39	0.68	0.75	0.50	0.38
	0.1	2.61	0.89	1.00	1.26	0.98^{ab}	3.21^{a}	0.33	0.58	0.73	1.01	0.47	0.46
	0	0.71	0.96	1.07	1.05	0.99^{ab}	0.87^{ab}	0.43	0.23	0.75	0.69	0.52	0.44
60	0.01	0.50	1.09	1.19	1.08	0.94^{ab}	1.03^{ab}	0.61	0.40	1.00	0.81	0.54	0.54
	0.1	1.88	0.47	0.92	1.17	0.87^{ab}	1.39^{ab}	0.33	0.51	0.65	0.86	0.46	0.32
SEM[2]		0.182	0.060	0.045	0.032	0.032	0.215	0.052	0.036	0.045	0.034	0.029	0.067
Main effects													
HB (%)													
0		0.52^{b}	0.67	0.91	1.17	1.00	0.73	0.71	0.50	0.88	0.93	0.70^{a}	0.63
30		1.57^{a}	0.68	0.92	1.13	0.94	1.88	0.42	0.42	0.80	0.86	0.52^{b}	0.53
60		1.03^{ab}	0.84	1.06	1.10	0.93	1.10	0.45	0.38	0.80	0.79	0.50^{b}	0.43
β-glucanase (%)													
0		0.54^{b}	0.76	0.92	1.07	0.99	0.63	0.49	0.29^{b}	0.94^{a}	0.82	0.60	0.57
0.01		0.89^{ab}	0.75	0.98	1.09	0.92	1.28	0.56	0.47^{ab}	0.87^{ab}	0.87	0.58	0.61
0.1		1.70^{a}	0.68	0.98	1.23	0.96	1.80	0.54	0.53^{a}	0.67^{b}	0.88	0.54	0.42
Probability													
HB		0.02	0.60	0.36	0.64	0.63	0.08	0.06	0.39	0.60	0.19	0.01	0.47
β-glucanase		0.01	0.86	0.80	0.09	0.61	0.03	0.85	0.01	0.02	0.75	0.60	0.51
HB × β-glucanase		0.09	0.07	0.52	0.15	0.01	0.03	0.46	0.74	0.14	0.11	0.96	0.36

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

[1]HB - hulless barley.

[2]SEM - pooled standard error of mean (n=12 birds per treatment).

Table 4. 14. Effects of hulless barley and β-glucanase on relative gene expression in caeca of broiler chickens

HB[1] (%)	BGase (%)	d 11				d 33			
		MUC2	MUC5ac	PCNA	MCT1	MUC2	MUC5ac	PCNA	MCT1
	0	0.23	0.43^{a}	0.27	0.19^{ab}	2.43	2.13	1.78	1.76
0	0.01	0.12	0.13^{b}	0.13	0.18^{ab}	1.48	1.03	1.59	1.21
	0.1	0.14	0.19^{ab}	0.20	0.24^{ab}	1.36	1.04	2.79	3.24
	0	0.10	0.13^{b}	0.17	0.13^{b}	1.88	1.45	1.80	2.47
30	0.01	0.22	0.32^{ab}	0.28	0.21^{ab}	1.51	1.09	2.34	1.87
	0.1	0.06	0.10^{b}	0.08	0.08^{b}	2.00	1.35	1.92	2.20
	0	0.32	0.32^{ab}	0.33	0.45^{a}	1.99	1.47	2.08	2.18
60	0.01	0.22	0.27^{ab}	0.25	0.23^{ab}	1.59	1.58	1.80	2.20
	0.1	0.16	0.20^{ab}	0.22	0.18^{ab}	2.11	1.57	2.08	2.17
SEM[2]		0.015	0.021	0.017	0.019	0.159	0.128	0.158	0.182
Main effects									
HB (%)									
0		0.16^{ab}	0.24	0.20	0.20	1.76	1.40	2.05	2.07
30		0.13^{b}	0.18	0.18	0.14	1.80	1.30	2.02	2.10
60		0.23^{a}	0.26	0.27	0.29	1.90	1.54	1.99	2.18
BGase (%)									
0		0.22^{a}	0.29	0.26	0.26	2.10	1.68	1.88	2.14
0.01		0.19^{ab}	0.24	0.22	0.21	1.53	1.23	1.91	1.76
0.1		0.12^{b}	0.16	0.17	0.16	1.82	1.32	2.26	2.45
Probability									
HB		0.01	0.18	0.12	0.01	0.90	0.85	0.96	0.93
BGase		0.02	0.03	0.12	0.16	0.24	0.11	0.34	0.10
HB × BGase		0.06	0.002	0.06	0.04	0.88	0.47	0.34	0.13

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

[1]HB - hulless barley; BGase - β-glucanase.

[2]SEM - pooled standard error of mean (n=12 birds per treatment).

4.4.7 Gastro-intestinal tract morphology

At d 11, relative GI segment empty weights, lengths, organ weights and content weights were affected by HB and BGase (Tables 4.15 and 4.16). 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. 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 heavier proventriculus and colon weights, and colon length in comparison to 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 caeca. The crop, duodenum and caecal interactions did not follow obvious trends. 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 the liver weight with the enzyme not affected 0 and 30% HB treatments, but the 0.1% BGase treatment resulted 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.

Gastro-intestinal tract size and content weights were affected by HB and BGase at d 33 (Tables 4.17 and 4.18). As seen at 11 d, the digestive tract empty weights (gizzard, duodenum, jejunum, small intestine, caeca and colon) and lengths (duodenum, small intestine, caeca and colon) increased with the addition of HB. 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, 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, caeca and colon were highest for the 60% HB diets, and except for the crop, lowest for the 0% HB diets. The addition of 0.1% BGase reduced the content weight of jejunum, ileum and colon compared to not adding an enzyme to the diet. Interaction was found for the content weight of small intestine, and the content weight was higher with 0.1% compared to 0% BGase for the birds fed 60% HB. Further, it increased with the increasing HB level in the diet. Dietary inclusion of 0.01% BGase yielded intermediate content weight values for these tissues. The liver and pancreas weights increased with the level of diet HB, while the liver weights were lower when BGase was included in the diet.

Table 4. 15. Effects of hulless barley and β-glucanase on gastro-intestinal tissue weights and lengths (proportional to body weight) of broiler chickens aged 11 days

HB[1]	BGase	Empty weight (%)									Length (cm/100g)					
(%)	(%)	Crop	Proven	Gizzard	Duo	Jejunum	Ileum	SI	Caeca	Colon	Duo	Jejunum	Ileum	SI	Caeca	Colon
	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
	0.1	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
	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
	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
	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
	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[2]		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^{ab}	1.85	6.10^{b}	0.55	0.22^{b}	6.30	14.89^{ab}	14.04	35.23^{b}	4.74	1.23^{ab}
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^{ab}	2.38	1.66	2.63	1.83	6.12	0.57	0.22^{ab}	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^{ab}
Probability																
HB		0.42	0.01	<.0001	0.29	0.01	0.23	<.0001	0.10	<.0001	0.08	0.01	0.25	0.001	0.11	0.01
BGase		0.14	0.01	0.16	0.67	0.64	0.47	0.06	0.99	0.01	0.43	0.06	0.83	0.26	0.78	0.05
HB × BGase		0.24	0.21	0.36	0.44	0.68	0.59	0.10	0.28	0.66	0.39	0.55	0.77	0.26	0.38	0.28

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

[1]HB - hulless barley; BGase - β-glucanase; Proven - proventriculus; Duo - duodenum; SI - small intestine.

[2]SEM - pooled standard error of mean (n=12 birds per treatment).

Table 4. 16. Effects of hulless barley and β-glucanase on gastro-intestinal content and organ weights as a percentage of body weight of broiler chickens aged 11 days

HB[1] (%)	BGase (%)	Content									Weight		
		Crop	Proventriculus	Gizzard	Duodenum	Jejunum	Ileum	SI	Caeca	Colon	Liver	Spleen	Pancreas
0	0	0.23^{c}	0.06	0.57	0.05^{ab}	0.44	0.36^{bc}	0.84^{bc}	0.11^{ab}	0.03	4.67^{a}	0.13	0.44
	0.01	0.44^{abc}	0.08	0.67	0.04^{b}	0.44	0.40^{bc}	0.87^{bc}	0.14^{a}	0.03	4.47^{ab}	0.12	0.47
	0.1	0.43^{abc}	0.05	0.59	0.04^{b}	0.40	0.28^{c}	0.71^{c}	0.06^{b}	0.02	4.46^{ab}	0.11	0.40
30	0	0.43^{abc}	0.17	0.80	0.04^{b}	0.46	0.38^{bc}	0.88^{bc}	0.09^{ab}	0.04	4.29^{ab}	0.12	0.45
	0.01	0.29^{abc}	0.11	0.80	0.04^{b}	0.39	0.32^{bc}	0.74^{bc}	0.06^{b}	0.04	4.41^{ab}	0.13	0.50
	0.1	0.28^{bc}	0.06	0.72	0.05^{ab}	0.41	0.36^{bc}	0.80^{bc}	0.09^{ab}	0.04	4.43^{ab}	0.10	0.46
60	0	0.48^{abc}	0.06	0.89	0.08^{a}	0.59	0.60^{a}	1.26^{a}	0.08^{ab}	0.06	4.12^{b}	0.13	0.54
	0.01	0.50^{ab}	0.05	0.75	0.03^{b}	0.49	0.43^{b}	0.95^{b}	0.06^{b}	0.06	4.49^{ab}	0.13	0.50
	0.1	0.54^{a}	0.05	0.81	0.05^{ab}	0.45	0.41^{bc}	0.89^{bc}	0.12^{ab}	0.04	4.61^{a}	0.11	0.50
SEM[2]		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^{ab}	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^{ab}
0.01		0.41	0.08	0.74	0.03	0.44^{ab}	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}
Probability													
HB		0.001	0.03	0.0002	0.09	0.002	<.0001	<.0001	0.18	0.0001	0.18	0.20	<.0001
BGase		0.73	0.23	0.64	0.03	0.01	0.0004	<.0001	0.76	0.02	0.23	0.002	0.01
HB × BGase		0.02	0.32	0.36	0.04	0.40	0.003	0.006	0.0007	0.44	0.003	0.78	0.12

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

Table 4. 17. Effects of hulless barley and β-glucanase on gastro-intestinal tissue weights and lengths (proportional to body weight) of broiler chickens aged 33 days

HB[1] (%)	BGase (%)	Empty weight (%)									Length (cm/100g)					
		Crop	Proven	Gizzard	Duo	Jejunum	Ileum	SI	Caeca	Colon	Duo	Jejunum	Ileum	SI	Caeca	Colon
	0	0.28	0.37	0.97	0.78	1.48	0.96	3.21	0.31	0.12	1.44	3.53^{bc}	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^{bc}	3.49^{b}	8.71	1.34	0.34
	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
	0	0.28	0.40	1.04	0.84	1.48	1.05	3.37	0.36	0.15	1.63	3.83^{bc}	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^{bc}	3.42^{b}	8.50	1.43	0.34
	0.1	0.29	0.37	1.10	0.82	1.46	0.99	3.27	0.37	0.13	1.55	3.70^{bc}	3.76^{b}	9.00	1.43	0.35
	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
	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[2]		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^{ab}	1.47^{b}	1.02	3.33^{b}	0.36^{a}	0.14^{b}	1.57^{ab}	3.69	3.68	8.94^{b}	1.49^{ab}	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^{ab}	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
Probability																
HB		0.50	0.14	0.001	0.03	0.002	0.14	0.002	0.001	0.001	0.003	0.001	0.001	<.0001	0.007	0.001
BGase		0.48	0.67	0.13	0.88	0.27	0.59	0.39	0.17	0.02	0.33	0.004	0.004	0.002	0.21	0.87
HB × BGase		0.76	0.62	0.97	0.84	0.85	0.20	0.48	0.97	0.12	0.34	0.01	0.05	0.01	0.66	0.38

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

[1]HB - hulless barley; BGase - β-glucanase; Proven - proventriculus; Duo - duodenum; SI - small intestine.

[2]SEM - pooled standard error of mean (n=18 birds per treatment).

Table 4. 18. Effects of hulless barley and β-glucanase on relative GI content weights and organ weights as a percentage of body weight of broiler chickens aged 33 days

HB[1] (%)	BGase (%)	Content									Weight		
		Crop	Proventriculus	Gizzard	Duodenum	Jejunum	Ileum	SI	Caeca	Colon	Liver	Spleen	Pancreas
	0	1.15	0.18	0.75	0.13	1.04	0.93	2.09^{bc}	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^{bc}	0.21	0.15	2.88	0.12	0.25
	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
	0	1.10	0.19	0.97	0.12	1.12	1.12	2.36^{abc}	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^{bc}	0.22	0.14	2.89	0.12	0.25
	0.1	1.08	0.06	1.05	0.14	1.01	1.04	2.19^{bc}	0.23	0.14	2.87	0.12	0.27
	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^{ab}	0.22	0.18	2.90	0.13	0.28
	0.1	1.44	0.06	1.33	0.09	0.86	0.97	1.91^{bc}	0.32	0.14	2.88	0.12	0.27
SEM[2]		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^{ab}	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^{ab}	0.15^{ab}	2.97^{ab}	0.12	0.26^{ab}
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^{ab}	2.22	0.22	0.16^{ab}	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
Probability													
HB		0.04	0.26	<.0001	0.08	0.18	<.0001	0.0006	0.04	0.001	0.03	0.60	0.0002
BGase		0.83	0.16	0.31	0.36	0.002	0.001	0.0004	0.28	0.02	0.004	0.96	0.45
HB × BGase		0.70	0.52	0.99	0.46	0.12	0.09	0.04	0.28	0.14	0.15	0.27	0.43

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

[1]HB - hulless barley; BGase - β-glucanase; SI - small intestine.

[2]SEM - pooled standard error of mean (n=18 birds per treatment).

4.5 Discussion

Performance variables of broiler chickens in this study were within a normal range according to Ross 308 Broiler Performance Objectives (Aviagen, 2014), and were affected by treatment in an age-dependent manner. Overall, performance variables decreased with increasing levels of HB, which is at least partially associated with the high level of fibre, and reduced energy and starch content in HB compared to 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 current study, which supports this hypothesis. In addition, comparatively lower nutrient digestibility might also be related to the poor performance in the broilers fed HB-based diets in comparison to wheat. The ileal viscosity was higher with the 60 than 0% HB in broilers given the diets with 0% BGase at d 33. Therefore, the increased ileal digesta viscosity might affect the digestibility of nutrients including fat, starch and protein (Edney et al., 1989; Friesen et al., 1992; Fuente et al., 1995; Mathlouthi et al., 2002a; Rodríguez et al., 2012), and in turn affect broiler performance (Jacob and Pescatore, 2014). The digestibility of fat affects more compared to the other nutrients with increasing level of HB, since it has been reported the high level of soluble fibre increases bile acid deconjugation through bacterial dysbiosis (Campbell et al., 1983; Feighner and Dashkevicz, 1988). Further, high fibre level in the HB cell wall might encapsulates nutrients including starch and protein and reduces the access to digestive enzyme and affects nutrient digestibility in chickens, since previous research observed a similar cage effect caused by wheat arabinoxylan (Masey-O'neill et al., 2014). The lower digestibility of HB compared to wheat-based diets was also supported by the higher digestive tract tissue weights (including pancreas weight) and lengths, and digesta content reported in this study, since GIT attempts to compensate for the nutrient digestion process by increasing GIT size and feed retention time (Salih et al., 1991; Brenes et al., 1993a; 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 d 0-11. In contrast, production performance increased with both 0.01 and 0.1% dosages of BGase, and the 0.1% BGase resulted in better performance compared to 0.01% BGase in broilers after d 11. The differences in treatment effects on performance do not appear to relate to digesta

viscosity since at both ages, viscosity was numerically lower for the 0.1 compared to the 0.01% BGase level. Further, this agrees with the finding that enzyme reduced Mp, Mw and MW-10% values in a similar dose-dependent manner at both ages. This may indicate that young and older broilers vary in their ability to effectively utilize lower molecular weight, fermentable carbohydrates. It is possible that the less mature digestive tract, and less diversified microbial population in young broiler chickens (Lu et al., 2003; Crhanova et al., 2011; Awad et al., 2016; Ocejo et al., 2019) was unable to effectively ferment the increased indigestible carbohydrate load, particularly in the disease state induced by coccidiosis challenge at five d of age. Maturation of the digestive tract with age (Dibner et al., 1996; Iji et al., 2001) and establishment of a beneficial and diverse GI 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), and it may be responsible for the production improvement with age. Adaptation of digestive tract microbiota to β-glucan with age is also probable. This type of age adaptation has been noted in broilers fed a wheat-based diet; an increased ability of digestive tract microbiota to degrade wheat arabinoxylan in the lower GIT was found with increasing broiler age (Bautil et al., 2019). The numerical reduction of ileal viscosity with the age of the chickens in this study and previous research (Petersen et al., 1999; Lee et al., 2017) further supports age dependent maturation of GIT microbiota, since the digesta viscosity inducing high MW, soluble β-glucan is degraded with the age of the birds. The effect of BGase on the performance and viscosity was significant at 60% HB level in the diets, and it is attributed to the higher content of β-glucan in the broiler diets at 60% HB compared to 0 and 30% HB levels.

The increased 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 d 11 and 33, and it might be associated with increased nutrient digestibility that leads to less requirement for a more extensive digestive tract. Further, the reduction of the pancreas weight with BGase addition to the diets also demonstrates BGase-mediated enhancement of nutrient digestibility as the requisite of pancreatic enzymes is less with the improvement of the digestion process in broiler chickens. The higher digestibility might be

related to the reduction of digesta viscosity or nutrient encapsulation by the endosperm cell wall (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 passage time which is associated with the reduction of digesta viscosity (Almirall and Esteve-Garcia, 1994; Salih et al., 1991). However, the only significant effect of BGase on viscosity was observed for the 60% HB diet 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. It is further supported by the absence of HB effect on viscosity at d 11. Hulless barley β-glucan might have undergone germination before the harvesting, and it may affect the digesta viscosity although β-glucan content was 8.70 and 0.74 in HB and wheat, respectively. Further, wheat might have consisted of a very high level of arabinoxylan, which contributes to high digesta viscosity in the GIT of broiler chickens (Choct and Annison, 1992). Furthermore, the disease status might affect the viscosity since the birds were challenged for coccidiosis at d five, and *Eimeria* may cause osmotic and absorptive changes in the host GIT that affects digesta viscosity (Crompton et al., 1976). A higher percentage of mortality (43.3% from the total mortality) was related to systemic infection or necrotic enteritis indicating the coccidiosis challenge was successful in broiler chickens.

Exogenous BGase induced fermentation of low MW β-glucan in the lower GIT of broilers was not obvious in the current study since the treatment effects were minimal on the ileal and caecal SCFA. Cereal β-glucan might increase broiler performance also via modulating immune system, since oat β-glucan affects immune function and digestive tract health (Estrada et al., 1997; Yun et al., 1997, Yun et al., 2003). Further, oat β-glucan increased the activation of Dectin-1 receptors of human dendritic cells, and immune capacity when the β-glucan was pre-treated with endo-glucanase (Sahasrabudhe et al., 2016). It is suggested that endo-glucanase treatment reduced the particle size and thereby increased the surface area of specific enzyme-binding sites, which emphasizes microbial enzyme-mediated β-glucan depolymerization in the digestive tract. However, research has not been conducted regarding the immune function of cereal β-glucan in chickens.

The MW of soluble β-glucan ileal digesta was affected by both cereal grain and the use of exogenous enzymes. Both Mp and Mw of β-glucan in the ileal digesta were higher when the birds were fed HB-based diets compared to a wheat-based diet without addition of BGase. This was not unexpected because HB contains higher MW β-glucan compared to wheat (Cui et al., 2000; Storsley et al., 2003). According to the literature, the β-glucan MW of barley and wheat ranges from 31 - 2700×10^3 and 209 - 416×10^3, respectively (Biliaderis and Izydorczyk, 2007).

Analysis of the diet β-glucan MW in the current study demonstrated a similar MW for HB with and without exogenous BGase (60% HB/0%BGase - Mp 762×10^3, Mw 648×10^3; 60%HB/0.1%BGase - Mp 758×10^3, Mw 624×10^3) suggesting little or no enzyme activity prior to feed consumption. Further, these values demonstrate β-glucan MW is reduced to a large degree in the ileal digesta, even without addition of BGase (Mp – 78×10^3, Mw - 80×10^3 with 60% HB) at d 11. There are several potential explanations for the MW reduction between diet and digesta in the current study. The digestive process including moistening, may lead to the activation of endogenous BGase from HB in the upper GIT of chickens (Ribeiro et al., 2011). Alternately, it may be due to the action of BGase derived from the microbes colonizing the upper chicken GIT (Cardoso et al., 2014; Józefiak et al., 2006). Grain cell walls have a complex structure and β-glucan is associated with other non-starch polysaccharides including heteroxylans, as well as protein and phenolic acids (Burton and Fincher, 2014). Bound together, these compounds might lead to a high β-glucan MW. However, cell walls may release β-glucan molecules with exposure to diet digestion in the chicken GIT, and therefore reduce the MW even without addition of exogenous BGase.

Molecular weight as indicated by Mp, Mw and MW-10% of β-glucan in the ileal digesta decreased with the addition of BGase when the birds were fed an HB-based diet. These data confirm the ability of BGase to depolymerize high MW β-glucan into low MW β-glucan in the GIT of chickens and demonstrate the degree and nature of the depolymerization associated with exogenous enzyme use. The reduction in MW with increasing level of BGase indicates the positive response of a very high dosage of enzyme compared to the standard dose (0.01% BGase) on β-glucan depolymerization at both ages of the broiler chickens. The higher response of 0.1% BGase level compared to the 0.01% BGase in terms of ileal soluble β-glucan

depolymerization might be associated with the relatively short transit time of digesta in chicken GIT and therefore less time for enzyme and substrate interaction (Hughes, 2008; Rougiere and Carre, 2010). Further, the optimum pH for BGase is 4.5, although it also has activity over a wider range of pH (Econase GT 200 P, 2019). This suggests more efficient enzyme action is restricted to certain GIT locations, which further reduces the availability of time for the enzyme to act on β-glucan. The reduction of MW-10% β-glucan molecules further supports the depolymerization of β-glucan, because it indicates the MW distribution curve has shifted towards the production of small β-glucan molecules due to the action of BGase and there is a greater amount of small MW carbohydrates available to undergo bacterial fermentation. The bimodal size distribution of Figure 4.2 shows two distinct peaks which indicates two distinct populations of β-glucan molecules when 0.1% BGase is added to the 60% HB diet; major peak associated with Mp and larger molecular weight peak, which was not found for the 0% BGase treatment (Figure 4.1). The reason for the larger peak is not obvious but might relate to aggregation of smaller β-glucan molecules, which has been previously shown to occur, particularly with increasing pH found in the distal small intestine (Gaborieau and Castignolles, 2011; Holtekjølen et al., 2014). It might also be associated with the release of insoluble β-glucan which had not yet been depolymerized.

There was a minimum or no BGase effect on MW parameters of the birds given wheat-based diets at both d 11 and 33. Wheat might be less susceptible to exogenous BGase in comparison to HB, which in turn might be associated with the structural differences in wheat and barley β-glucan. The ratios of cellotriosyl to cellotetraosyl units (DP3/DP4) in wheat and barley β-glucan are 3.0-4.5 and 2.3-3.4, respectively. The proportions of trisaccharides (DP3) and tetrasaccharides (DP4) in wheat and barley are DP3 - 67-72%, DP4 - 21-24% and DP3 - 52-69, DP4 - 25-33%, respectively (Biliaderis and Izydorczyk, 2007). Therefore, wheat β-glucan has a more regular structure in comparison to barley. Furthermore, low and high DP3/DP4 ratio β-glucans are more uniform causing increased aggregation and reduced β-glucan solubility (Burton and Fincher, 2014), which possibly results in reduced susceptibility to feed BGase.

Overall, MW parameters in this study were lower (on average – Mp - 19.2%; Mw - 11.1%; Mw-10% - 16.5% reduction) in broilers aged 33 d compared to 11 d. This reduction may

be associated with the adaptation of the digestive tract microbiota to the diets with increasing age (Bautil et al., 2019) and the ability of the gut microbiota of the older birds to secrete more non-starch polysaccharidases including BGase. It is possible that this type of adaptation is also related to the lower ileal viscosity at d 33 compared to d 11.

Microbial fermentation products (SCFA) in the lower GIT were assessed to determine the effect of exogenous BGase on providing low MW, soluble HB β-glucan as substrates to increase carbohydrate fermentation in broilers, and GI pH was determined as an indication of the changes in microbial fermentation. Dietary treatments had only minor effects on ileal SCFA levels in this study and levels did not relate to ileal pH. Major SCFA did not change with treatment, whereas both increasing levels of HB and BGase resulted in increased ileal pH. In contrast, caecal SCFA concentrations (total and major SCFA) at d 11 increased with the highest level of BGase, but only at the 60% HB level, which is related to the caecal pH since the caecal pH decreased with BGase at the highest HB level. It has been observed carbohydrate metabolizing microbes are abundant in chicken caeca compared to other categories of microbiota (Qu et al., 2008; Danzeisen et al., 2011), and it might be associated with more significant treatment effects in the caeca over the ileum in the broilers especially at d 11. However, it is difficult to conclude BGase effect on increasing carbohydrate fermentation in the caeca based on the treatment effect only for one treatment (60% HB, 0.1% BGase) in broiler chickens. There is little previous research that examined the BGase effect on SCFA production in broilers fed barley, and the results were inconsistent, and demonstrated a minimum enzyme effect (Józefiak et al., 2005; 2006). Nevertheless, the results do not demonstrate SCFA production since the digesta samples of broilers were collected only at a point in each collection and the digesta levels relate to the balance between production and utilization by bacteria or the host, as well as frequency of ileum or caecal evacuation. A portion of SCFA might get absorbed into the portal circulation before the digesta samples were collected in the study, and it might be expected that gene expression of SCFA transporters would increase with increasing SCFA levels. However, MCT1 expression in the ileum decreased with increasing HB, which is in accordance with ileal pH increasing with HB level. Trans-epithelial SCFA transportation via MCT1 in ruminants is proton-mediated active transport (Halestrap and Meredith, 2004; Kirat et al., 2006). However, transportation of

SCFA across the intestinal epithelium might be associated with other mechanisms including passive diffusion in chickens. Therefore, the relative expression of MCT1 might not indicate total SCFA transportation across the intestinal epithelium in chickens.

Caecal pH decreased with the addition of BGase to the 60% HB-based diet (only at d 11), which is an indication of increased carbohydrate fermentation because of the high availability of low MW β-glucan originating from high MW β-glucan depolymerization. In contrast, the ileal pH increased with HB and BGase, but these treatments had no effect on SCFA at both ages. Further, there was a trend (P = 0.08) for the interaction of HB and BGase on ileal pH at d 11, showing increased pH with increasing HB and BGase levels. According to the β-glucan MW distribution data, BGase resulted in an increased amount of low MW soluble β-glucan in the ileum which might be fermentable. However, the feed passage rate may have increased in the ileum with the reduction of soluble β-glucan MW, and therefore less time is available for the bacterial fermentation in the ileum. Consequently, low MW material may enter the caeca, and increase bacterial fermentation. It is supported by the reduction of mean retention time of stomach in the growing pigs with increasing nutrient solubility in the diets (Schop et al., 2019). However, many other factors contribute to intestinal pH including protein and minerals in the diet. Increased protein fermentation in the lower GIT of chickens increases intestinal pH due to the protein fermentation metabolites including ammonia, phenol, indole, and biogenic amines (Apajalahti, 2005). Minerals in the diet also help in buffering the acidity in GIT that results in increased pH.

Undigested nutrients and GI metabolic products including SCFA activate the ileal brake by increasing the production of GI hormones including GLP-1 and PYY from L cells (Gee et al., 1996; Aoki et al., 2017). The hormones decrease feed intake, and GI motility by enhancing satiety, and thereby increasing feed passage time (Stanley et al., 2004; Maljaars et al., 2008). Further, it has been observed high levels of low MW β-glucan in diets caused greater production of SCFA, and also increased secretion of GLP1 and PYY in mice (Miyamoto et al., 2018). In the current study, the relative empty weights and lengths of GI segments increased with HB but not appeared to have an association with SCFA induced gut hormone secretion in broilers, since the relative weights and lengths of GIT components did not increase with BGase supplementation,

and there were very minor treatment effects on the estimated carbohydrate fermentation in broilers according to the results of SCFA concentrations and GI pH. The current study used a purified form of BGase, which is different from the previous research (Józefiak et al., 2005; 2006) that used both BGase and endoxylanase. Thus, xylanase affects depolymerization of arabinoxylan, which might affect carbohydrate fermentation of broilers more in comparison to the individual use of BGase in the diet. Therefore, it appears small MW soluble β-glucan of HB did not exert its' prebiotic effect through increased carbohydrate fermentation. However, there might be other prebiotic mechanisms of barley β-glucan including the modification of GI microbial population, immune system modulation and competitive exclusion of pathogenic microorganisms in GIT of broiler chickens.

Gastro-intestinal wall histomorphology is an indication of GI health in chickens and increased epithelial integrity of the GIT wall is associated with improved nutrient digestion, absorption, and GI health in chickens (Choct, 2009; Onrust et al., 2015). However, there were few treatment effects on the histo-morphological parameters in the current study. Short chain fatty acids, and in particular butyrate, which result from carbohydrate fermentation in chickens positively affect GI epithelial integrity as shown by measurements including villi height and width, crypt depth and goblet cell distribution (Liu et al., 2017; Wu et al., 2018). Villi height decreased with the highest level of HB compared to wheat, and it might be due to high MW β-glucan, and the resulting high viscosity, which damage epithelial villi in the ileum. Previous research has found feeding high levels of soluble non-starch polysaccharides to chickens causes a reduction of villi height (Rakowska et al., 1993). Crypt depth in the ileum increased with the highest level of BGase, which is an indication of epithelial growth, which might be due to the beneficial effect of butyrate. Addition of dietary sodium butyrate increased intestinal villi height and goblet cell numbers (Wu et al., 2018), crypt depth (Antongiovanni et al., 2007; Panda et al., 2009), and villi height to crypt depth ratio (Hu and Guo, 2007) in broilers. However, butyrate concentration in the ileum did not increase with dietary BGase in the current study, but this may relate to the aforementioned inaccuracies of measuring SCFA levels. The number and distribution of goblet cells in the ileum was not affected by the treatment, however the ileal MUC2 expression was lower with the highest level of BGase compared to the control. This

finding may relate to BGase-mediated improvement of broiler immune defence mechanisms, which results in less requirement of mucin to combat against pathogens (Kufe, 2009). In addition, HB increased the expression of ileal MUC2, and this might relate to an increase in the front-line epithelial defence mechanism since HB mediated high ileal viscosity can increase the colonization of pathogenic microbes in the digestive tract of chickens (Hansson and Johansson, 2010).

It is concluded feed BGase causes depolymerization of high MW β-glucan in HB into low MW β-glucan in a dose-dependent manner in the GIT of chickens. The resulting low MW β-glucan increased production performance in older birds, although no improvement, or a reduction of performance was observed for young broilers aged less than 11 d when the highest level of BGase was fed. Conversely, there were very minor effects of HB and BGase on the GI physiological and histo-morphological measurements of broiler chickens. Except for an increase of caecal SCFA concentrations with 0.1% BGase at 60% HB fed 11 d-old broilers, HB and BGase did not increase carbohydrate fermentation (as indicated by SCFA levels), despite the exogenous BGase dependent depolymerization of high MW β-glucan of HB at both ages. There might be other mechanisms including cereal β-glucan induced immune function, which affects the growth performance and GIT morphological and physiological characteristics in broiler chickens using depolymerized small MW β-glucan of HB, which needs further investigation.

5.0 EFFECTS OF EXOGENOUS BETA-GLUCANASE ON ILEAL SOLUBLE DIGESTA BETA-GLUCAN MOLECULAR WEIGHT, DIGESTIVE TRACT CHARACTERISTICS AND PERFORMANCE OF BROILER CHICKENS FED HULLESS BARLEY-BASED DIETS WITH AND WITHOUT MEDICATION

5.1 Abstract

Limited use of medication in poultry feed has led to the investigation of feed additives including exogenous enzymes as alternatives to antibiotics for controlling enteric disease. The objective of this study was to evaluate the effects of diet β-glucanase (BGase) and medication on β-glucan depolymerization, digestive tract characteristics and performance of broilers using 2 experiments. In both trials, broilers were fed a high β-glucan hulless barley (HB) based diet with BGase (Econase GT 200P from ABVista; 0 and 0.1%) and medication (Bacitracin and Salinomycin Na; with and without) arranged as a 2 × 2 factorial. In Experiment 1, 160 broilers were housed in battery cages from d 0 to 28. Each treatment was assigned to 10 cages (4 birds in each cage) and samples were collected at d 28. In Experiment 2, a total of 2376 broilers were housed in floor pens and challenged for coccidiosis at d 5. Each treatment was assigned to 9 floor pens in each of 9 broiler rooms (66 mixed-sex birds per pen). Performance was assessed at d 11, 22 and 33. Samples were collected at d 11 and 33. In Experiment 1, β-glucan weight average molecular weight (Mw) in ileal digesta was lower with medication in the treatments without BGase. Peak molecular weight (Mp) and Mw were lower with the use of BGase regardless of medication. The maximum molecular weight for the smallest 10% β-glucan (MW-10%) was lower with BGase. In Experiment 2, Mp was lower with medication in broilers fed diets without BGase. Diet BGase resulted in lower Mp regardless of medication, and the degree of response was lower with medication. The MW-10% was lower with BGase despite antibiotic addition. Both diet medication and BGase resulted in higher ileal pH. Overall, BGase resulted in lower

caecal pH. Ileal SCFA concentrations except butyric acid were higher with BGase at d 33. Caecal SCFA concentrations were higher with BGase in the treatments without medication at d 11. They were lower with medication for the treatments with BGase. Further, total SCFA and acetic acid in the caeca were lower with medication at d 33. Body weight gain (BWG) and feed efficiency were higher with medication regardless of the BGase use through-out the trial (except d 11-22 feed efficiency). Overall, BGase resulted in higher BWG after d 11, and lower and higher feed efficiency before and after d 11, respectively, in the treatments without medication. In conclusion, diet BGase and medication depolymerized soluble ileal β-glucan in broilers. Exogenous BGase increased carbohydrate fermentation and appeared as a partial replacement for diet medication to increase growth performance of coccidiosis challenged broilers.

Keywords: antibiotics, ionophores, prebiotics, oligosaccharides, fermentation

5.2 Introduction

Antibiotics have been added to poultry diets to prevent enteric diseases and to enhance the performance of broiler chickens (Engberg et al., 2000). However, with the regulatory agency implemented removal of specific antibiotics (European Commission, 2005; Chicken Farmers of Canada, 2019) and increased public awareness regarding emergence of antibiotic-resistant genes (Diarra et al., 2007; Furtula et al., 2010; Forgetta et al., 2012; Garcia-Migura et al., 2014; Roth et al., 2019), usage of in-feed antibiotics has decreased in the poultry industry. Therefore, the investigation of alternatives to antibiotics is a major focus to control infectious enteric disease (Diarra and Malouin, 2014). Potential alternatives to antibiotics which have been studied include probiotics, prebiotics, organic acids, essential oils and feed enzymes (Huyghebaert et al., 2011; Ducatelle et al., 2015; Gadde et al., 2017).

Prebiotics are non-digestible feed ingredients that beneficially affect the host by selectively stimulating the growth and function of beneficial microbiota in the digestive tract (Gaggia et al., 2010). The most commonly available prebiotics are oligosaccharides from various sources, and small molecular weight (MW) polysaccharides derived from cereal grains. Studies in the literature have focused on molecules such as fructo-oligosaccharides, mannose-

oligosaccharides, xylo-oligosaccharides and arabinoxylo-oligosaccharides in terms of improving poultry digestive tract health and production performance, and modulating intestinal microbiota, epithelial integrity, and immune function in poultry. Dietary mannan-oligosaccharides have been shown to increase morphological development of the digestive tract and colonization of beneficial bacteria, while reducing pathogenic bacteria in chickens (Baurhoo et al., 2007; 2009). Fructo-oligosacchaides has also demonstrated beneficial effects on broiler chickens in terms of intestinal epithelial morphology (Xu et al., 2003; Shang et al., 2015), digestive tract microbiota (Flickinger et al., 2003; Xu et al., 2003; Boguslawska-Tryk et al., 2012) and bird immune response (Kim et al., 2011; Khodambashi Emami et al., 2012). Dietary inclusion of arabinoxylo-oligosaccharides/ xylo-oligosaccharides affect gastro-intestinal (GI) microbial populations of chickens by increasing beneficial bacteria including bifidobacteria, Lactobacilli and *Clostridium* cluster XIV (Courtin et al., 2008b; De Maesschalck et al., 2015), and reducing *Salmonella* colonization in the caeca and translocation to the spleen (Eeckhaut et al., 2008). In addition, exogenous xylanase in wheat-based diets increased the number of GI beneficial bacteria including lactic acid bacteria, while reducing pathogenic bacteria in broiler chickens (Owens et al., 2008; Rodríguez et al., 2012), probably by decreasing the MW of soluble arabinoxylan derived from the wheat. Arabinoxylan has been extensively studied concerning their ability to act as a prebiotic since arabinoxylan is found in the cell walls of the most common cereals used in poultry feed (wheat and corn). However, research is limited regarding cereal β-glucan since it predominates in barley and oats, which are less commonly found in poultry feed. Therefore, it is relevant to investigate the prebiotic effect of low MW barley β-glucan produced by supplementing exogenous β-glucanase (BGase) to a barley-based broiler chicken diet.

Hulless barley (HB) contains a high level of large MW β-glucan compared to conventional barley due to the removal of the hull during harvesting (Classen et al., 1985; McNab and Smithard, 1992). Further, many HB cultivars are developed for the human food industry, and as a result are selected for high β-glucan content (Ames et al., 2006). Dietary enzymes such as endo-β-glucanase depolymerize larger MW β-glucan producing lower MW compounds, which are fermentable in the distal digestive tract (Józefiak et al., 2005). A consequence of fermentation is the production of short-chain fatty acids (SCFA), which are

thought to improve digestive tract morphology and physiology, and stimulate the establishment of beneficial bacterial populations, while at the same time reducing colonization by pathogens (Józefiak et al., 2010; Jiang et al., 2015). However, the effects of exogenous BGase on microbial fermentation and digestive tract physiology and morphology are less-well studied, and the results were inconsistent in previous research. Therefore, investigation of the mechanism of action of diet BGase on HB β-glucan might contribute to the understanding of the enzyme effect on digestive tract characteristics of chickens.

The mechanisms of action of medication in feed are not clear, although antibiotics have been successfully used to promote growth and feed efficiency, and improve bird health (Engberg et al., 2000; Khodambashi Emami et al., 2012). The primary mechanism is generally accepted to be modulation of the diversity and relative abundance of the digestive tract microbial community, and thereby the control of enteric disease and immune function of broiler chickens (Dibner and Richards, 2005; Torok et al., 2011; Lee et al., 2012; Singh et al., 2013), but other beneficial mechanisms are possible. Investigating the interaction between medication and enzyme use in high fibre diets offers potential to add knowledge on medication mechanisms of action and to study the effectiveness of enzymes in reducing the negative effects of enteric disease.

The objective of the current study was to investigate the effects of exogenous BGase and medication on ileal soluble digesta β-glucan MW distribution, digestive tract characteristics, and production performance of broiler chickens fed an HB-based diet. It was hypothesized that exogenous BGase will depolymerize high MW β-glucan, resulting in increased fermentation in the distal digestive tract and beneficial effects on the digestive tract morphology and physiology, and production performance of broiler chickens and thereby reduce the requirement of medication in broilers fed HB-diets.

5.3 Materials and methods

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

The study utilized the same experimental design and treatments in two different environments. Experiment 1 was completed in battery cages and a low disease challenge environment, while Experiment 2 was completed in litter floor pens using broilers challenged for coccidiosis at 5 d of age. In addition, data collection was performed at two points (d 11 and 33) in Experiment 2 to compare birds in an infective (d 11) and a recovered stage (d 33). The rationale for these experiments was to determine if treatments produce the same effects in the prescribed settings.

5.3.1 Birds and housing

Experiment 1. A total of 160 broiler chickens (Ross × Ross 308) obtained from a commercial hatchery were housed in battery cages. The dimensions of the cages were 51 cm in length, 51 cm in width and 46 cm in height. The grid size of the wire mesh floor of each cage was 2.54 × 2.54 but was covered by a 1.27 × 1.27 cm mesh until d 7. There were two levels of battery cages that were in two rows with back to back cages. The starting room temperature was 32°C and it was gradually decreased by 2.8°C per week. The minimum light intensity was 25 lux during the experimental period, and the day length was 23 h (d 0-7) and 18 h (d 8-28). Birds were given feed and water *ad-libitum* throughout the experiment. Each cage had a front-mounted feed trough (51 cm in length) and two height adjustable nipple drinkers. Extra feed and water were supplied to the birds from d 0 to 5 using supplementary chick feeders (50 cm long, plastic) and icecube trays (16 cell), respectively. There were 10 cage replications per treatment and 4 birds per cage. Treatments were randomly assigned to the battery cages.

Experiment 2. A total of 2376 one d old male and female (Ross × Ross 308) broiler chickens were obtained from a commercial hatchery and randomly placed in 36 floor pens (2.3 m × 2.0 m) in nine environmentally controlled rooms. Each room contained four pens randomly assigned to the four treatments; each treatment was replicated nine times. Each pen contained a

tube feeder (pan diameter - 36 cm from 0 to 25 d and 43 cm thereafter) and a height-adjustable nipple drinker (6 Lubing nipples). Additional feed and water were supplied to each pen using a cardboard egg tray and an ice cube tray, respectively for the first week. Straw was placed in each room at a thickness of 7.5-10 cm. The room temperature was 33°C at the beginning of the experiment and was gradually reduced to 21°C by d 25. Day length was gradually reduced from 23 h at d 0 to 17 h at d 12. Light intensity was set to 20 lux at the start of the experiment and gradually decreased to 10 lux by d 10. Birds were given feed and water *ad-libitum* throughout the experiment.

5.3.2 Experimental diets

The dietary treatments were arranged according to a 2 × 2 factorial arrangement (BGase and medication) in both experiments. Beta-glucanase (Econase GT 200 P from ABVista, Wiltshire, UK) levels were 0 and 0.1% (β-glucanase activity of 0 and 200,000 BU/kg, respectively), and diets were fed without or with medication (Bacitracin (Zoetis Canada Inc., Kirkland, QC, Canada) at 4.4 mg/kg and Salinomycin Sodium (Phibro Animal Health Corporation, Teaneck, NJ) at 25 mg/kg). Diets were based on 60% hulless barley (CDC Fibar) and were formulated to meet or exceed Ross 308 broiler nutrition specifications. The ingredients and calculated nutrient levels are shown in Tables 5.1. Diets were fed in crumble form in Experiment 1. In Experiment 2, starter diets (d 0-11) were fed in crumble form and grower diets (d 11-33) were given initially in crumble form, and then switched to a pellet form. The pelleting temperature was controlled between 70-75°C to prevent high temperature-induced BGase inactivation during feed processing. Measured β-glucanase activity in diets approached the estimated values in both experiments, thereby confirming β-glucanase was added correctly, and that activity was not lost during feed processing. Xylanase activity was non-detectable in experimental diets.

Table 5. 1. Ingredients and calculated nutrient levels (%) of Experimental diets

Ingredient	Experiment 1	Experiment 2	
		Starter	Grower
Hulless barley	60.00	59.09	60.00
Wheat	4.46	0.00	4.55
Soybean meal	26.93	32.97	26.99
Canola oil	4.07	3.29	4.13
Monocalcium phosphate	1.20	1.40	1.20
Limestone	1.52	1.64	1.52
Sodium chloride	0.38	0.43	0.38
Vitamin-mineral broiler premix[1]	0.50	0.50	0.50
Choline chloride	0.10	0.10	0.10
DL-Methionine	0.27	0.30	0.27
L-Threonine	0.05	0.07	0.05
L-Lysine HCl	0.22	0.21	0.22
Nutrient, calculated			
AME (kcal/kg)	3100	3000	3100
Crude protein	21.24	23.46	21.24
Crude fat	5.57	4.74	5.57
Calcium	0.87	0.96	0.87
Chloride	0.36	0.38	0.36
Non-phytate phosphorous	0.44	0.48	0.44
Potassium	0.83	0.92	0.83
Sodium	0.18	0.20	0.18
Digestible arginine	1.35	1.50	1.35
Digestible isoleucine	0.81	0.90	0.81
Digestible leucine	1.47	1.61	1.47
Digestible lysine	1.15	1.28	1.15
Digestible methionine	0.54	0.60	0.54
Digestible methionine and cysteine	0.87	0.95	0.87
Digestible threonine	0.77	0.86	0.77
Digestible tryptophan	0.24	0.27	0.24
Digestible valine	0.87	0.96	0.87

[1]Vitamin-mineral premix provided the following per kilogram of complete diet: vitamin A, 11,000 IU; vitamin D_3, 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 3773 mg.

5.3.3 Coccidiosis challenge

In Experiment 2, all the birds were challenged with the Coccivac B-52 live vaccine (Merck Animal Health; 1.3× recommended dose). The vaccination was completed at d 5 to facilitate uniform intake of coccidian oocysts by the birds. The vaccine contains oocysts of *Eimeria acervulina, E. mivatis, E. maxima* and *E. tenella*. The vaccine was sprayed on feed located in a cardboard egg tray and into water placed in an ice cube tray. A 30 cm wide Kraft brown paper strip (Model S-8511S, ULINE Canada, Milton, Ontario, Canada) was placed under the full length of the nipple drinker line in each pen before vaccination to facilitate oocyst ingestion by the birds. In addition, 60% relative humidity was maintained in the rooms, using humidifiers and water application, to facilitate oocyst cycling. Feeders and drinkers were raised in each pen before vaccination, and they were put-down once the vaccine containing feed and water was consumed by the birds.

5.3.4 Performance data collection

Body weight and feed intake (FI) were measured on a cage basis at d 7, 14, 21 and 28 in Experiment 1. In Experiment 2, body weight and FI were measured on a pen basis at d 11, 22 and 32. Mortality was recorded daily, and dead birds were sent to Prairie Diagnostic Services for a detailed necropsy.

5.3.5 Sample collection

Excreta collection. In Experiment 1, excreta were collected at d 26 and 27 on a cage basis at 12 h intervals for 36 h (3 times) using aluminum trays placed under each cage. Samples were pooled on a cage basis at each collection and stored at -20°C. Excreta samples were later dried using a forced air oven (55˚C) and pooled by replication.

Tissue and digesta collection. In Experiment 1, all the birds were euthanized on d 28, whereas in Experiment 2, a total of four birds per pen were euthanized at two collection points (d 11 and 33) by administering T-61 (Embutramide, Mebezonium iodide and tetracaine; Merck animal health, Kirkland, Quebec, Canada) into the brachial vein. Birds were weighed individually. Two birds were used for pH measurement and to collect samples for SCFA analysis and histology (in Experiment 2 only) at each collection. *In-situ* pH of crop, gizzard, duodenum,

jejunum, ileum, caeca and colon contents was measured using a Beckman Coulter 34 pH meter (Model PHI 34, Beckman Instruments, Fullerton, CA). Two 1 cm samples of mid-ileum were sectioned and placed in 10% neutral buffered formalin and stored at room temperature to analyze for GIT histo-morphology. Total ileal and caecal contents were collected into plastic centrifuge tubes and stored at -20°C for the analysis of SCFA. Two birds were used to collect digestive tract size, content, and organ data at each collection in both trials. The digestive tract was detached from the bird carcass and then sectioned into the crop, proventriculus, gizzard, duodenum, jejunum, ileum, caeca, and colon; the liver, spleen and pancreas were removed and weighed. Full and empty weights of all sections and the length of each intestinal section were recorded. The content weight of each section was determined by subtracting empty weight from the full weight. Relative tissue weights and lengths were calculated based on individual bird weight. Total ileal contents were collected into plastic snap-cap vials (pooled from all the birds in a cage in Experiment 1; only 1 bird in Experiment 2) and centrifuged for 3-5 min at 14,000 rpm using a Beckman microfuge (Model E 348720, Beckman Instruments, INC, Palo Alto, CA). Then the viscosity of ileal supernatant was measured using a Brookfield digital viscometer (Model LVDV-III, Brookfield Engineering Labs, INC, Stoughton, MA 02072). The rest of the ileal supernatant was stored at -80°C for β-glucan MW distribution analysis.

5.3.6 Nutritional analysis of experimental diets and excreta

Experimental diets and ingredients (HB and wheat) were ground to 1 mm (for N, fat, ash, minerals, and insoluble and soluble dietary fibre analysis) and 0.5 mm (for β-glucan and total starch analysis) screen-hole sizes using a Retsch laboratory mill (Retsch ZM 200, Germany). Beta-glucan was analyzed (AOAC Method 995.16, AACC Method 32-23 and ICC Standard Method No. 168) using a Megazyme analysis kit (Mixed-linkage beta-glucan assay procedure/McCleary method, Megazyme International Ireland Ltd., Bray Business Park, Bray, Co. Wicklow, Ireland). Moisture was analyzed using AOAC method 930.15. Nitrogen was analyzed using a Leco nitrogen analyzer (Model Leco-FP-528L, Leco Corporation, St. Joseph, MA, USA), and 6.25 was the N to CP conversion factor. Insoluble and soluble dietary fibre were analyzed using a Megazyme kit (Total dietary fibre assay procedure, Megazyme International Ireland Ltd., Bray Business Park, Bray, Co. Wicklow, Ireland) according to the AOAC method

991.43 and AACC method 32-07.01, and total dietary fibre (TDF) was obtained by adding IDF and SDF. Ether extraction was completed using Goldfish Extraction Apparatus (Labconco model 35001; Labconco, Kansas, MO, USA) following the AOAC method 920.39 to determine fat content. Ash content was analyzed according to AOAC method 942.05 using a muffle oven (Model Lindberg/Blue BF51842C, Asheville, NC 28804, USA). In addition, diets and ingredients were analyzed for total starch using AOAC method 996.11 and AACC method 76-13.01 using a Megazyme kit (Total starch assay procedure, Amyloglucosidase/α-amylase method, Megazyme International Ireland Ltd., Bray Business Park, Bray, Co. Wicklow, Ireland). Further, mineral analysis was completed on ingredients by SGS Agrifood Laboratories (Guelph, ON, Canada) following methods using AOAC methods 985.01. In addition, treatment diets from experiments 1 and 2 were analyzed for β-glucanase (EC 3.2.1.6) and xylanase activity (EC 3.2.1.8) according to the AB Vista methods of ESC Standard Analytical Methods SAM042-01 and SAM038, respectively (ABVista, Wiltshire, UK).

5.3.7 Beta-glucan molecular weight distribution

Ileal supernatant samples collected from both experiments were boiled for 15 min to inactivate endogenous BGase activity. The sample was then analyzed for β-glucan MW using size exclusion chromatography and calcofluor post-column derivatization (Boyd et al., 2017). The two columns used for HPLC were Shodex OHpak SB-806M with OHpak SB-G column guard and a Waters Ultrahydrogel linear column. The mobile phase was 0.1M Tris buffer (pH=8). Molar mass distribution curves were used to obtain β-glucan peak molecular weight (Mp), average molecular weight (Mw) and the maximum molecular weight for the smallest 10% β-glucan molecules (MW-10%) of each sample. Peak molecular weight is the molecular weight of the most abundant β-glucan proportion. Weight average molecular weight (Mw) is the average of the molecular weights of all β-glucan molecules (considering the weight fraction of each type of molecule).

5.3.8 Short chain fatty acids analysis

Short chain fatty acids were analyzed in triplicate according to the procedure described by Zhao et al. (2006) with minor changes. The internal standard for the analysis was made up of 20 ml of 25% phosphoric acid, 300 µl of isocaproic acid and deionized water. Three hundred

microliters of acetic acid, 200 µl of propionic acid, 100 µl of butyric acid and 50 µl of isobutyric, isovaleric, valeric, caproic and lactic acids were used to make the standard solution. The digesta was thawed and mixed with 25% phosphoric acid at 1:1 and kept at room temperature for 10 min with occasional shaking. It was then centrifuged at 12,000 rpm for 10 min. The supernatant (1 ml) was mixed with 1 ml of the internal standard, and centrifuged at 16,000 rpm for 10 min. It was filtered using a 0.45-micron nylon filter, and the filtrate was placed in a GC autosampler vial and injected into a Zebron Capillary Gas Chromatography column (length 30m, internal diameter 0.25 mm, film thickness 0.25 µm; (Zebron^{TM}ZB-FFAP, Phenomenex, Torrance, CA). The SCFA analysis was completed using at Thermo Scientific Gas Chromatography system (Model Trace 1310, Milan, Italy).

5.3.9 Histomorphology of gastro-intestinal wall

In Experiment 2, ileal tissue samples preserved in 10% formalin buffered saline were cut into two longitudinal sections and embedded in paraffin. Two slides were made from each sample to obtain GIT morphology measurements (Hematoxylin and Eosin stain) and goblet cell (GC) categorization (Alcian Blue/ Periodic Acid-Schiff stain). An Optika B-290TB digital microscope (Bergamo, Italy) was used to observe slides, and an HDCE-X3 digital camera with Optika Vision Lite software was used to capture the images. Well-oriented 8-10 villi and crypts per section were used to measure villi length, width, and crypt depth. Villi length was considered as the length from the tip of a villus to the villus-crypt junction, and the villi width was measured at the middle of the villus height. The depth of the invagination between adjacent villi was considered as the crypt depth. Goblet cells were counted around the perimeter of 8-10 well-oriented villi and crypts per section and the three categories of GC were identified, acidic mucin-producing GC (stained in blue), neutral mucin-producing GC (stained in magenta) and mixed mucin-producing GC (stained in purple) (Osho et al., 2017).

5.3.10 Statistical analysis

Data were analyzed using the Proc Mixed model of the SAS 9.4 (SAS 9.4, Carey, N.C. 2008). Both experiments were randomized complete block designs, and the battery cage level and room were considered as blocks for Experiments 1 and 2, respectively. Treatments were replicated 10 times in Experiment 1 (battery cages equally distributed in two levels), and nine

times in Experiment 2 (one pen in nine different rooms). Differences were considered significant when $P \leq 0.05$. Data were checked for normality and analyzed using 2-way ANOVA. Tukey-Kramer test was used to detect significant differences between means.

5.4 Results

5.4.1 Ingredient nutrient composition

In experiment 1, TDF, IDF, SDF and total β-glucan in HB were 29.0, 19.6, 9.6 and 8.70%, respectively, and the same parameters were 15.2, 13.7, 1.6 and 0.68%, respectively for wheat. The content of total starch, CP, fat, and ash were measured as 49.7, 16.2, 2.4 and 2.4%, respectively, in HB, and as 64.1, 15.0, 1.2 and 1.9% in wheat. In experiment 2, TDF, IDF, SDF and total β-glucan were 26.7, 18.9, 7.8 and 8.70% (HB); 14.4, 12.4, 2.0 and 0.64% (wheat), respectively. In addition, total starch, CP, fat, and ash were determined to be 53.7, 16.2, 2.8 and 2.4% in HB, and as 62.8, 14.9, 1.2 and 1.7% in wheat, respectively.

5.4.2 Beta-glucan molecular weight distribution

In Experiment 1, both Mp and Mw were affected by the interaction between main effects; values were lower with enzyme use regardless of diet medication, but the degree of response was less in medicated diets. In addition, Mw was lower with the use of medication when the birds were given diets without BGase. The MW-10% values were unaffected by medication but were lower with 0.1% compared to 0% BGase.

In Experiment 2, interactions were found for all molecular weight criteria at both ages (11 and 33 d) except for Mw at 11 d, which was also unaffected by medication or BGase. Values for Mp and Mw-10% followed a similar trend, with enzyme consistently reducing values, but with the degree of response less in medicated diets. In the absence of enzyme, medication reduced Mp at both ages and MW-10% on d 33. The interaction for Mw at 33 d was due to enzyme decreasing and increasing Mw for nonmedicated and medicated diets, respectively. Molecular weight parameters were numerically lower in Experiment 1 compared to Experiment 2.

Figures 5.1A and 5.1B compare the β-glucan MW distribution of ileal digesta from broilers fed diets without medication, and without and with BGase, respectively (Experiment 2). Beta-glucanase increased the proportion of low MW β-glucan as shown by curve placement relative to the blue line at x-axis point $1e^4$. Further, a bimodal size distribution is shown in Figure 5.1B; in addition to Mp, a larger molecular weight fraction also results from BGase use. Diet medication also increased the proportion of low MW β-glucan in comparison to the nonmedicated diet and this is contrasted in Figures 5.1C and 5.1A.

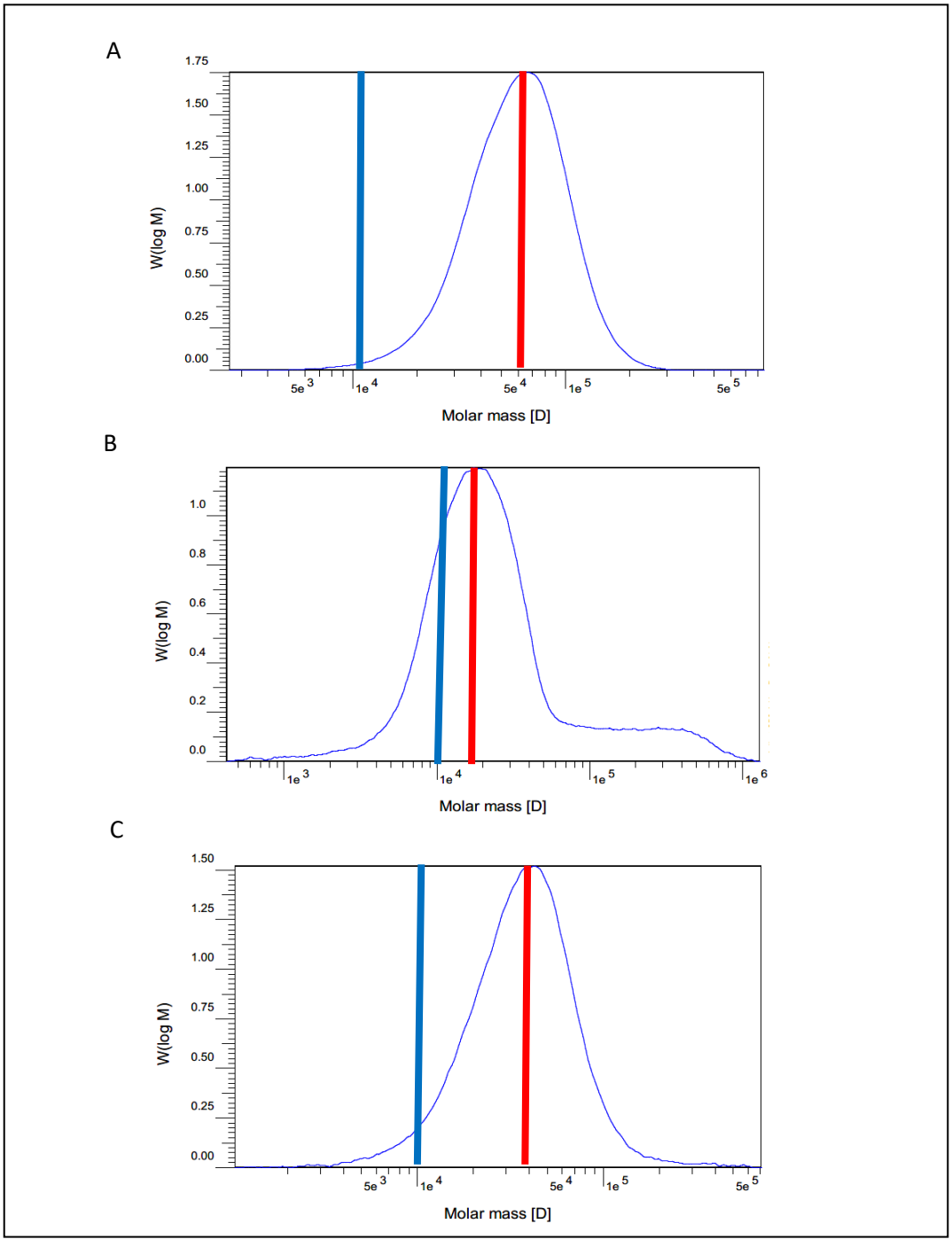

Figure 5. 1. Beta-glucan molecular weight distribution in soluble ileal digesta from broilers fed 60% hulless barley diets. Blue lines denote point 1e4 on the x-axis and red lines indicate the Mp of the distribution curve. A. Without medication, 0% β-glucanase; B. Without medication, 0.1% β-glucanase; C. With medication, 0% β-glucanase

Table 5. 2. Effects of diet medication and β-glucanase on β-glucan molecular weight in ileal content of broiler chickens

Medication	β-glucanase (%)	Molecular weight (g/mol)								
		Experiment 1			Experiment 2					
		d 28			d 11			d 33		
		Mp[1]	Mw	MW-10%	Mp	Mw	MW-10%	Mp	Mw	MW-10%
without	0	19799[a]	36199[a]	6096	78293[a]	80971	33322[a]	65176[a]	69508[a]	29025[a]
	0.1	7793[b]	8434[c]	1955	24568[c]	63835	7250[b]	16985[c]	48316[b]	7074[c]
with	0	16824[a]	19119[b]	5326	54475[b]	59002	26065[a]	40595[b]	49017[b]	13586[b]
	0.1	10401[b]	9929[c]	2201	27677[c]	61898	10586[b]	22144[c]	60641[a]	8157[c]
SEM[2]		1148.1	2513.9	509.2	5982.7	3537.4	2717.0	4481.7	2258.9	1890.1
Main effects										
Medication										
without		13796	22317	4025	51431	72403	20286	41080	58912	18049
with		13612	14524	3763	41076	60450	18325	31370	54829	10871
β-glucanase (%)										
0		18311	27659	5711[a]	66384	69986	29694	52885	59263	21305
0.1		9096	9181	2078[b]	26122	62867	8918	19565	54479	7615
Probability										
Medication		0.86	0.001	0.70	0.08	0.06	0.39	0.04	0.16	<.0001
β-glucanase		<.0001	<.0001	<.0001	<.0001	0.21	<.0001	<.0001	0.10	<.0001
Medication × β-glucanase		0.01	0.0004	0.45	0.03	0.09	0.03	0.004	<.0001	<.0001

[a-c]Means within a main effect or interaction not sharing a common superscript are significantly different ($P \leq 0.05$).
[1]Mp - peak MW; Mw - weighted average MW; MW-10% - The maximum MW for the smallest 10% molecules.
[2]SEM - pooled standard error of mean (d 28, n=6 cages per treatment; d 11 and 33, n=6 birds per treatment).

5.4.3 Viscosity

Ileal digesta viscosity was not affected by medication in Experiment 1 but was reduced with the use of BGase. In Experiment 2 at 11 d, an interaction was found between medication and BGase; BGase reduced viscosity without dietary medication. In the interaction, the highest viscosity was noted for the treatment without medication or BGase, and the lowest were the treatments with BGase; treatment with medication and without BGase was the intermediate. At d 33 in Experiment 2, BGase decreased viscosity, but there was no medication effect.

Table 5. 3. Effects of diet medication and β-glucanase on the ileal soluble digesta viscosity of broiler chickens

Medication	β-glucanase (%)	Viscosity (cP)		
		Experiment 1	Experiment 2	
		d 28	d 11	d 33
without	0	4.72	9.73[a]	3.98
	0.1	3.33	3.53[b]	2.30
with	0	4.16	6.04[ab]	4.61
	0.1	3.38	4.13[b]	2.80
SEM[1]		0.147	0.674	0.250
Main effects				
Medication				
without		4.02	6.63	3.14
with		3.77	5.08	3.70
β-glucanase (%)				
0		4.44[a]	7.89	4.29[a]
0.1		3.35[b]	3.83	2.55[b]
Probability				
Medication		0.25	0.11	0.17
β-glucanase		<.0001	0.0005	0.0002
Medication × β-glucanase		0.16	0.03	0.86

[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 (d 28; n=10 cages per treatment/ d 11; n=6 birds per treatment/ d 33; n=9 birds per treatment).

5.4.4 Short chain fatty acids and gastro-intestinal pH

Ileal digesta SCFA levels were not affected by dietary treatments in Experiment 1, except for caproic acid concentration, where values were lower with BGase supplementation (Table 5.4). Similarly, caecal digesta SCFA concentrations and molar percentages were also not affected by treatment (Table 5.5). Noteworthy, the interaction between medication and BGase tended to be significant (P = 0.06-0.09) for the concentrations of total and individual SCFA. In all cases, levels tended to decrease with enzyme use in the non-medicated diets and increase with enzyme use in the medicated diets.

To a large extent, dietary treatment did not affect ileal digesta SCFA of 11d old broilers in Experiment 2 (Table 5.6). The exception was a significant interaction between medication and BGase for valeric acid. Without medication, levels of valeric acid decreased with enzyme use, while levels increased with enzyme use when the medication was included in the diet. A similar trend (P = 0.10) was noted for isovaleric acid. Levels of caproic acid decreased with the enzyme use. Interactions between the main effects were found for the molar percentage of valeric, isovaleric (P = 0.06) and caproic acids. In diets without medication, BGase did not affect acid concentration. When the medication was used, BGase increased acid levels. Dietary treatment interactions were also noted for the proportional levels of propionic and lactic acids. All mean differences were small and often not significant, but values tended to increase and decrease with BGase use in nonmedicated and medicated diets, respectively.

The interactions between medication and BGase use at 33 d in Experiment 2 were significant for total and individual caecal digesta SCFA (Table 5.7). The concentrations were higher with 0.1 compared to 0% BGase in the birds given diets without medication. However, BGase did not affect SCFA concentrations in the treatments with medication. Concentrations for birds fed medicated diets were lower than those fed un-medicated diets for the treatments with BGase. The molar percentages of propionic and isobutyric acids were decreased by medication, while enzyme use decreased and increased the proportions of acetic and butyric acids, and valeric acid, respectively. The interaction between main effects was significant for the proportional isovaleric levels, with enzyme tending to decrease levels in unmedicated diets and

increase levels in medicated diets. Although the above effects were significant, differences were small.

Medication and the interactions between medication and BGase did not affect the concentrations and molar percentages of ileal SCFA at d 33 in Experiment 2 (Table 5.8). All ileal SCFA concentrations except butyric acid were higher because of BGase use. In addition, the percentages of valeric and isovaleric acids were higher for the 0.1 compared to the 0% BGase treatment. In contrast, the lactic acid percentage was slightly lower with enzyme use.

Main effect interactions were not found for the concentrations and molar percentages of caecal digesta SCFA at d 33 (Table 5.9). However, the concentrations of total SCFA and acetic acid were lower in medicated diets. Similarly, all other SCFA levels except butyric acid tended (P = 0.06-0.07) to be lower with medication use. The molar percentages of acetic acid decreased, while butyric, valeric (P = 0.08) and isovaleric (P = 0.09) acids increased with medication use. Enzyme use decreased the molar percentage of acetic acid, and increased values for all other SCFA except butyric acid.

Except for the duodenum, medication, BGase and their interactions did not affect digestive tract pH in Experiment 1(Table 5.10). Enzyme use increased duodenal pH from 6.08 to 6.20. Main effect interactions were not found for digestive tract pH in Experiment 2, except for caecal pH at d 11 (Table 5.11); pH was lower with the enzyme use, but only in the diets without medication. Medication resulted in higher pH in the crop at d 11, and the ileum at both d 11 and 33. Duodenal and ileal pH was higher with the use of BGase at d 11. Gizzard and caecal pH were lower, and the ileal pH was higher with the addition of diet BGase at d 33.

Table 5. 4. Effects of diet medication and β-glucanase on ileal digesta short chain fatty acids of broiler chickens at 28 days of age (Experiment 1)

Medication	BGase[1] (%)	SCFA µmol/g of wet ileal content									Molar percentage of total SCFA							
		Total	Ace	Pro	But	Isob	Val	Isov	Cap	Lac	Ace	Pro	But	Isob	Isov	Val	Cap	Lac
without	0	165.8	61.8	22.2	10.6	2.7	3.3	2.9	1.3	60.6	37.5	13.1	6.4	1.6	1.7	1.9	0.7	36.6
	0.1	157.2	59.1	20.8	10.3	2.9	2.6	2.2	1.0	58.0	37.6	13.3	6.5	1.8	1.4	1.6	0.6	36.9
with	0	173.5	66.4	23.4	10.8	2.5	2.7	2.9	1.5	63.0	38.3	13.2	6.3	1.4	1.6	1.5	0.8	36.5
	0.1	156.9	59.1	21.8	10.3	2.4	2.6	2.6	1.2	56.5	37.6	14.0	6.6	1.4	1.6	1.6	0.8	36.1
SEM[2]		4.51	1.66	0.75	0.31	0.18	0.17	0.17	0.07	1.60	0.23	0.28	0.09	0.10	0.09	0.08	0.09	0.24
Main effects																		
Medication																		
Without		161.5	60.5	21.5	10.4	2.8	2.9	2.6	1.1	59.3	37.6	13.2	6.5	1.7	1.5	1.7	0.7	36.7
With		165.2	62.7	22.6	10.5	2.4	2.6	2.8	1.3	59.8	38.0	13.6	6.4	1.4	1.6	1.5	0.8	36.3
BGase (%)																		
0		169.6	64.1	22.8	10.7	2.6	3.0	2.9	1.4[a]	61.8	37.9	13.2	6.3	1.5	1.7	1.7	0.8	36.6
0.1		157.0	59.1	21.3	10.3	2.6	2.6	2.4	1.1[b]	57.2	37.6	13.6	6.6	1.6	1.5	1.6	0.7	36.5
Probability (%)																		
Medication		0.66	0.46	0.41	0.86	0.31	0.38	0.55	0.10	0.87	0.38	0.48	0.80	0.13	0.78	0.25	0.08	0.38
BGase		0.13	0.11	0.28	0.53	0.94	0.28	0.11	0.02	0.13	0.57	0.45	0.27	0.55	0.34	0.55	0.10	0.85
Medication × BGase		0.63	0.45	0.94	0.90	0.67	0.36	0.52	0.73	0.51	0.40	0.59	0.61	0.57	0.34	0.23	0.35	0.47

[a-d]Means within a main effect or interaction not sharing a common superscript are significantly different ($P \leq 0.05$).
[1]BGase - β-glucanase; SCFA - short chain fatty acids; Ace - Acetic acid; Pro - Propionic acid; But - Butyric acid; Isob - Isobutyric acid; Val - Valeric acid; Isov - Isovaleric acid; Cap - Caproic acid.
[2]SEM - pooled standard error of mean (n=20 birds per treatment).

Table 5. 5. Effects of diet medication and β-glucanase on caecal short chain fatty acids of broiler chickens aged 28 days (Experiment 1)

Medication	BGase[1] (%)	SCFA µmol/g of wet caecal content								Molar percentage of total SCFA						
		Total	Ace	Pro	But	Isob	Val	Isov	Cap	Ace	Pro	But	Isob	Val	Isov	Cap
without	0	284.2	166.6	58.5	28.0	9.9	8.6	8.6	3.7	58.7	20.5	9.8	3.5	3.0	3.0	1.3
	0.1	273.9	161.7	56.5	27.0	8.4	8.3	8.3	3.5	59.0	20.6	9.9	3.0	3.0	3.0	1.3
with	0	267.5	158.0	55.2	26.2	8.2	8.1	8.1	3.5	59.0	20.6	9.8	3.0	3.0	3.0	1.3
	0.1	310.3	183.1	64.0	30.6	9.5	9.3	9.4	4.0	58.9	20.6	9.8	3.0	3.0	3.0	1.3
SEM[2]		7.59	4.49	1.60	0.74	0.35	0.23	0.23	0.10	0.23	0.28	0.09	0.10	0.08	0.09	0.03
Main effects																
Medication																
without		279.0	164.1	57.5	27.5	9.1	8.4	8.5	3.6	58.8	20.5	9.8	3.3	3.0	3.0	1.3
with		288.9	170.5	59.6	28.4	8.8	8.7	8.8	3.7	59.0	20.6	9.8	3.0	3.0	3.0	1.3
BGase (%)																
0		275.8	162.3	56.8	27.1	9.0	8.3	8.4	3.6	58.8	20.5	9.8	3.3	3.0	3.0	1.3
0.1		292.1	172.4	60.2	28.8	8.9	8.8	8.9	3.8	59.0	20.6	9.9	3.0	3.0	3.0	1.3
Probability (%)																
Medication		0.50	0.46	0.50	0.53	0.69	0.51	0.49	0.48	0.57	0.57	0.90	0.30	0.62	0.49	0.47
BGase		0.27	0.25	0.27	0.23	0.85	0.31	0.30	0.30	0.57	0.65	0.48	0.27	0.94	0.92	0.95
Medication × BGase		0.07	0.09	0.08	0.06	0.06	0.08	0.08	0.08	0.47	0.71	0.99	0.28	0.61	0.76	0.84

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

[1]BGase - β-glucanase; SCFA - short chain fatty acids; Ace - Acetic acid; Pro - Propionic acid; But - Butyric acid; Isob - Isobutyric acid; Val - Valeric acid; Isov - Isovaleric acid; Cap - Caproic acid.

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

Table 5. 6. Effects of diet medication and β-glucanase on ileal short chain fatty acids of broiler chickens aged 11 days (Experiment 2)

Medication	BGase[1] (%)	SCFA µmol/g of wet ileal content								Molar percentage of total SCFA						
		Total	Ace	Pro	But	Val	Isov	Cap	Lac	Ace	Pro	But	Val	Isov	Cap	Lac
without	0	125.3	48.2	18.4	8.2	2.7^{a}	1.5	1.19	44.9	38.4	14.6^{ab}	6.5	2.1^{a}	1.2	0.9^{a}	35.8^{ab}
	0.1	122.5	47.6	18.3	8.1	1.5^{bc}	1.4	0.79	44.6	38.8	14.9^{a}	6.6	1.2^{ab}	1.1	0.9^{a}	36.4^{ab}
with	0	121.5	46.8	18.0	7.6	1.3^{c}	1.4	1.19	45.1	38.6	14.8^{ab}	6.2	1.1^{b}	1.1	0.6^{b}	36.9^{a}
	0.1	118.7	45.3	17.2	7.7	2.5^{ab}	2.5	1.10	42.1	38.2	14.5^{b}	6.5	2.1^{a}	2.1	0.9^{a}	35.4^{b}
SEM[2]		1.93	0.71	0.28	0.22	0.17	0.19	0.05	0.84	0.21	0.05	0.13	0.13	0.15	0.03	0.17
Main effects																
Medication																
without		123.9	47.9	18.3	8.2	2.1	1.4	0.99	44.8	38.6	14.8	6.6	1.7	1.1	0.7	36.1
with		120.6	46.1	17.6	7.6	1.9	1.9	1.14	43.6	38.4	14.6	6.4	1.6	1.6	0.9	36.2
BGase (%)																
0		123.4	47.5	18.2	7.9	2.0	1.4	1.19^{a}	45.0	38.5	14.7	6.4	1.6	1.1	0.9	36.4
0.1		120.6	46.4	17.7	7.9	2.0	1.9	0.95^{b}	43.4	38.5	14.7	6.6	1.7	1.6	0.7	35.9
Probability (%)																
Medication		0.29	0.16	0.16	0.24	0.53	0.17	0.10	0.45	0.64	0.22	0.42	0.69	0.12	0.02	0.89
BGase		0.43	0.41	0.39	0.99	0.98	0.17	0.01	0.30	0.94	0.79	0.54	0.77	0.13	0.01	0.16
Medication × BGase		0.99	0.75	0.50	0.90	0.0003	0.10	0.09	0.39	0.36	0.01	0.84	0.002	0.06	0.04	0.001

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

[1]BGase - β-glucanase; SCFA - short chain fatty acids; Ace - Acetic acid; Pro - Propionic acid; But - Butyric acid; Isob - Isobutyric acid; Val - Valeric acid; Isov - Isovaleric acid; Cap - Caproic acid.

[2]SEM - pooled standard error of mean (n=12 birds per treatment).

Table 5. 7. Effects of diet medication and β-glucanase on caecal short chain fatty acids of broiler chickens aged 11 days (Experiment 2)

Medication	BGase[1] (%)	SCFA µmol/g of wet caecal content								Molar percentage of total SCFA						
		Total	Ace	Pro	But	Isob	Val	Isov	Cap	Ace	Pro	But	Isob	Val	Isov	Cap
without	0	228.6^{b}	134.1^{b}	49.7^{b}	22.7^{b}	7.4^{b}	4.3^{b}	7.4^{b}	2.7^{b}	58.6	21.8	9.9	3.2	1.7	3.3^{a}	0.1
	0.1	306.6^{a}	176.5^{a}	66.3^{a}	30.0^{a}	9.9^{a}	9.7^{a}	9.8^{a}	4.2^{a}	57.5	21.6	9.7	3.2	3.1	3.2^{ab}	0.1
with	0	172.8^{b}	100.9^{bc}	36.4^{bc}	17.5^{b}	5.4^{c}	4.6^{b}	5.4^{c}	2.3^{b}	58.3	21.1	10.1	3.1	2.7	3.1^{b}	0.1
	0.1	171.2^{b}	98.8^{c}	36.7^{c}	16.8^{b}	5.5^{c}	5.4^{b}	5.4^{c}	2.2^{b}	57.7	21.4	9.8	3.2	3.1	3.2^{ab}	0.1
SEM[2]		12.94	7.41	2.83	1.25	0.42	0.58	0.41	0.19	0.21	0.05	0.13	0.01	0.13	0.15	0.03
Main effects																
Medication																
without		267.6	155.3	58.0	26.3	8.7	7.0	8.6	3.4	58.1	21.7^{a}	9.8	3.2^{a}	2.4	3.2	0.1
with		172.0	99.8	36.6	17.2	5.4	5.0	5.4	2.3	58.0	21.3^{b}	9.8	3.1^{b}	2.9	3.1	0.1
BGase (%)																
0		200.7	117.5	43.1	20.1	6.4	4.5	6.4	2.5	58.5^{a}	21.5	10.0^{a}	3.2	2.2^{b}	3.2	0.1
0.1		238.9	137.7	51.5	23.4	7.7	7.5	7.6	3.2	57.6^{b}	21.5	9.8^{b}	3.2	3.1^{a}	3.1	0.1
Probability (%)																
Medication		<.0001	<.0001	<.0001	<.0001	<.0001	0.02	<.0001	0.0002	0.68	0.01	0.09	0.01	0.14	0.01	0.57
BGase		0.02	0.03	0.01	0.04	0.01	0.001	0.01	0.01	0.0004	0.91	0.01	0.89	0.007	0.93	0.34
Medication × BGase		0.01	0.02	0.02	0.01	0.02	0.01	0.03	0.005	0.22	0.17	0.64	0.08	0.16	0.05	0.38

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

[1]BGase - β-glucanase; SCFA - short chain fatty acids; Ace - Acetic acid; Pro - Propionic acid; But - Butyric acid; Isob - Isobutyric acid; Val - Valeric acid; Isov - Isovaleric acid; Cap - Caproic acid.

[2]SEM - pooled standard error of mean (n=12 birds per treatment).

Table 5. 8. Effects of diet medication and β-glucanase on ileal short chain fatty acids of broiler chickens aged 33 days (Experiment 2)

Medication	BGase[1] (%)	SCFA µmol/g of wet ileal content								Molar percentage of total SCFA						
		Total	Ace	Pro	But	Val	Isov	Cap	Lac	Ace	Pro	But	Val	Isov	Cap	Lac
without	0	115.2	44.6	17.0	7.6	1.5	1.6	1.0	41.6	38.7	14.79	6.6	1.3	1.4	0.8	36.1
	0.1	125.0	47.8	18.1	8.1	2.6	2.7	1.1	44.3	38.2	14.52	6.5	2.1	2.1	0.9	35.4
with	0	118.9	46.0	17.5	7.8	1.7	1.9	1.0	42.7	38.7	14.74	6.6	1.4	1.6	0.8	35.9
	0.1	123.0	47.1	17.9	7.5	2.6	2.6	1.1	43.8	38.3	14.60	6.1	2.1	2.1	0.9	35.6
SEM[2]		1.21	0.46	0.17	0.13	0.11	0.11	0.02	0.43	0.21	0.05	0.13	0.13	0.15	0.03	0.17
Main effects																
Medication																
without		120.1	46.2	17.6	7.8	2.1	2.1	1.0	42.9	38.5	14.6	6.5	1.7	1.7	0.9	35.7
with		120.9	46.5	17.7	7.7	2.2	2.3	1.0	43.2	38.5	14.6	6.3	1.8	1.8	0.9	35.7
BGase (%)																
0		117.0[b]	45.3[b]	17.2[b]	7.7	1.6[b]	1.7[b]	1.0[b]	42.1[b]	38.7	14.7	6.6	1.4[b]	1.5[b]	0.8	36.0[a]
0.1		124.0[a]	47.5[a]	18.0[a]	7.8	2.6[a]	2.6[a]	1.1[a]	44.0[a]	38.3	14.5	6.3	2.1[a]	2.1[a]	0.9	35.5[b]
Probability (%)																
Medication		0.73	0.72	0.69	0.51	0.71	0.48	0.88	0.70	0.91	0.88	0.30	0.77	0.53	0.82	0.91
BGase		0.003	0.02	0.02	0.68	<.0001	<.0001	0.01	0.02	0.30	0.10	0.12	0.001	0.003	0.18	0.001
Medication × BGase		0.22	0.24	0.34	0.15	0.61	0.40	0.92	0.34	0.76	0.25	0.37	0.83	0.57	0.72	0.24

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

[1]BGase - β-glucanase, SCFA - short chain fatty acids; Ace - Acetic acid; Pro - Propionic acid; But - Butyric acid; Isob - Isobutyric acid; Val - Valeric acid; Isov - Isovaleric acid; Cap - Caproic acid.

[2]SEM - pooled standard error of mean (n=18 birds per treatment).

Table 5. 9. Effects of diet medication and β-glucanase on caecal short chain fatty acids of broiler chickens aged 33 days (Experiment 2)

Medication	BGase[1] (%)	SCFA µmol/g of wet caecal content								Molar percentage of total SCFA						
		Total	Ace	Pro	But	Isob	Val	Isov	Cap	Ace	Pro	But	Isob	Val	Isov	Cap
without	0	225.0	132.2	46.5	22.5	6.9	6.8	6.8	2.9	58.8	20.6	10.0	3.0	3.04	3.05	1.31
	0.1	230.7	134.9	48.1	23.0	7.2	7.1	7.1	3.0	58.5	20.8	9.9	3.1	3.08	3.09	1.33
with	0	209.8	122.6	43.5	21.4	6.5	6.4	6.4	2.7	58.4	20.7	10.2	3.1	3.07	3.07	1.32
	0.1	215.5	125.3	45.1	22.0	6.7	6.6	6.7	2.8	58.1	20.9	10.2	3.1	3.10	3.11	1.33
SEM[2]		3.78	2.17	0.82	0.38	0.12	0.12	0.12	0.05	0.21	0.05	0.13	0.01	0.13	0.15	0.03
Main effects																
Medication																
without		227.8[a]	133.5[a]	47.3	22.7	7.0	6.9	7.0	3.0	58.6[a]	20.7	10.0[b]	3.1	3.06	3.07	1.32
with		212.6[b]	124.0[b]	44.3	21.7	6.6	6.5	6.5	2.8	58.2[b]	20.8	10.2[a]	3.1	3.08	3.09	1.33
BGase (%)																
0		217.4	127.4	45.0	22.0	6.7	6.6	6.6	2.8	58.6[a]	20.7[b]	10.1	3.0[b]	3.05[b]	3.06[b]	1.31[b]
0.1		223.1	130.1	46.6	22.5	6.9	6.8	6.9	2.9	58.3[b]	20.9[a]	10.0	3.1[a]	3.09[a]	3.09[a]	1.33[a]
Probability (%)																
Medication		0.04	0.02	0.06	0.15	0.06	0.07	0.07	0.07	0.005	0.20	0.02	0.14	0.08	0.09	0.12
BGase		0.43	0.51	0.31	0.50	0.27	0.29	0.28	0.27	0.03	0.02	0.75	0.004	0.01	0.01	0.004
Medication × BGase		0.99	0.99	0.99	0.93	0.98	0.99	0.99	0.94	0.93	0.97	0.85	0.88	0.93	0.97	0.59

[a-d]Means within a main effect or interaction not sharing a common superscript are significantly different ($P \leq 0.05$).
[1]BGase - β-glucanase; SCFA - short chain fatty acids; Ace - Acetic acid; Pro - Propionic acid; But - Butyric acid; Isob - Isobutyric acid; Val - Valeric acid; Isov - Isovaleric acid; Cap - Caproic acid.
[2]SEM - pooled standard error of mean (n=18 birds per treatment).

Table 5. 10. Effects of diet medication and β-glucanase on gastro-intestinal pH of broiler chickens at day 28 (Experiment 1)

Medication	β-glucanase (%)	Crop	Gizzard	Duodenum	Jejunum	Ileum	Caeca	Colon
without	0	5.29	3.54	6.05	5.99	7.08	6.02	6.92
	0.1	5.23	3.26	6.19	6.01	7.26	6.04	7.17
with	0	5.43	3.23	6.10	5.96	7.25	5.90	7.08
	0.1	5.20	3.17	6.21	6.05	7.27	5.93	7.13
SEM[1]		0.070	0.071	0.027	0.024	0.048	0.055	0.067
Main effects								
Medication								
without		5.26	3.40	6.12	5.99	7.17	6.03	7.04
with		5.31	3.20	6.16	6.00	7.26	5.91	7.11
β-glucanase (%)								
0		5.36	3.39	6.08[b]	5.97	7.16	5.96	7.00
0.1		5.21	3.22	6.20[a]	6.03	7.26	5.98	7.15
Probability								
Medication		0.70	0.15	0.46	0.89	0.25	0.29	0.61
β-glucanase		0.29	0.21	0.01	0.16	0.20	0.82	0.22
Medication × β-glucanase		0.55	0.41	0.80	0.40	0.29	0.94	0.43

[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 (n=20 birds per treatment).

Table 5. 11. Effects of diet medication and diet on gastro-intestinal pH of broiler chickens (Experiment 2)

Medication	[1]BGase (%)	pH											
		d 11						d 33					
		Crop	Gizzard	Duodenum	Jejunum	Ileum	Caeca	Crop	Gizzard	Duodenum	Jejunum	Ileum	Caeca
without	0	4.78	2.81	5.88	5.91	6.29	6.36[a]	4.94	3.67	6.15	5.93	6.50	6.22
	0.1	4.62	2.41	5.99	5.92	6.61	5.78[b]	4.84	3.44	6.01	5.99	6.94	6.03
with	0	4.93	2.49	5.90	5.90	6.62	5.70[b]	5.01	3.75	6.18	5.97	7.20	6.19
	0.1	5.09	2.55	6.06	6.01	6.97	5.77[b]	4.91	3.28	6.18	5.99	7.39	5.96
SEM[2]		0.052	0.057	0.024	0.018	0.053	0.061	0.052	0.057	0.024	0.018	0.053	0.061
Main effects													
Medication													
without		4.70[b]	2.61	5.94	5.92	6.45[b]	6.07	4.89	3.55	6.08	5.96	6.72[b]	6.12
with		5.01[a]	2.52	5.98	5.96	6.80[a]	5.74	4.96	3.52	6.18	5.98	7.30[a]	6.08
BGase (%)													
0		4.85	2.65	5.89[b]	5.91	6.45[b]	6.03	4.97	3.71[a]	6.16	5.95	6.85[b]	6.21[a]
0.1		4.86	2.48	6.03[a]	5.97	6.79[a]	5.78	4.87	3.36[b]	6.09	5.99	7.17[a]	5.99[b]
Probability													
Medication		0.001	0.41	0.33	0.25	0.0001	0.001	0.46	0.71	0.09	0.61	<.0001	0.65
BGase		0.97	0.12	0.004	0.10	0.0002	0.01	0.29	0.001	0.22	0.28	0.0007	0.04
Medication × BG		0.10	0.04	0.66	0.14	0.84	0.002	0.98	0.24	0.21	0.61	0.16	0.88

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

[1]BGase - β-glucanase.

[2] SEM - pooled standard error of mean (d 11; n=12 birds per treatment, d 33; n=18 birds per treatment).

5.4.5 Gastro-intestinal wall histomorphology

Gastrointestinal wall histology was examined only at d 11 and 33 in Experiment 2 (data not shown). Treatment effects were not prevalent nor consistent between ages. At d 11, medication decreased the crypt depth (137 vs 114 μm), while β-glucanase decreased the villi width (102 vs 90 μm). At 33 d, medication increased the number of acidic (70 versus 89) and decreased the number of mixed (8 vs 4) goblet cells per villus. Medication also increased the villi height to crypt depth ratio (4.5 vs 5.2).

5.4.6 Gastro-intestinal tract morphology

In Experiment 1, interactions were not found between BGase and medication for empty weights and lengths of digestive tract sections, except for crop weight (Table 5.12). Crop weight was lower with enzyme use when the birds were fed a non-medicated diet, but enzyme had no effect when the diets were medicated. Both ileum and colon weights were lower when enzyme was fed. Crop content weight was higher and duodenal and ileal content weights were lower when 0.1% BGase was fed (Table 5.13). Interactions were found for the content weights of the gizzard and jejunum. The gizzard content weight tended to be higher and lower with enzyme use in birds fed non-medicated and medicated diets, respectively. Beta-glucanase resulted in lower jejunal and small intestinal content weights in the absence of dietary antibiotics, but had no effect when medication was used.

In Experiment 2, interactions were found between medication and BGase for the proportional empty weights of the duodenum, jejunum, small intestine, and caeca at d 11 (Table 5.14). For all segments, feeding diets without medication or enzyme resulted in the heaviest weights. Using an enzyme in nonmedicated diets reduced the segment weights (jejunum and full small intestine), while enzyme use in diets with medication did not affect empty weight. Feeding an enzyme reduced the proventriculus empty weight. The length of the jejunum, ileum, small intestine, and caeca were shorter with medication use. The dietary enzyme reduced the length of the jejunum and the small intestine. The content weight of the small intestine was lower with the addition of BGase to the diets without medication. Medication reduced the content weight of the crop and caeca, while BGase reduced the content weight of the gizzard, jejunum, ileum and

colon. Diet medication reduced the pancreas weight, and diet enzyme increased the liver weight, and decreased the pancreas weight.

Diet medication decreased the proportional empty weights of the duodenum, jejunum, ileum, small intestine, and colon, and decreased the lengths of the same digestive tract segments in 33 d old broilers (Table 5.16). Dietary BGase resulted in lower empty weights for the crop, ileum, and small intestine; enzyme also reduced the lengths of the duodenum and ileum. Interactions between main effects were found for the empty weight of the jejunum, and the lengths of the jejunum and small intestine. For the interactions, enzyme use resulted in smaller tissues when non-medicated diets were fed, but had no effect when diets contained medication. Medication resulted in smaller digestive tract segments in these interactions.

The content weights of the duodenum and colon decreased with the use of BGase (Table 5.17). Medication similarly decreased the content weight of the duodenum. Interactions between medication and enzyme were found for the content weights of the gizzard ($P = 0.06$), jejunum, ileum, small intestine, and colon ($P = 0.06$). For the jejunum, ileum and colon segments, enzyme reduced weights in non-medicated diets, but did not affect content weights in the presence of medication. For gizzard content weights, enzyme increased and decreased values in diets without and with medication, respectively. An interaction was also found for the liver weight. The largest weight was found for the birds fed diets with no medication or enzyme; the addition of enzyme to the unmedicated diet resulted in lower weight, and the liver weights for medicated diets were smallest and unaffected by the enzyme in the diet.

Table 5. 12. Effects of diet medication and β-glucanase on gastro-intestinal tissue weights and lengths (proportional to body weight) of broiler chickens at d 28 (Experiment 1)

Medication	BGase[1] (%)	Empty weight (%)									Length (cm/100g)					
		Crop	Proven	Gizzard	Duo	Jejunum	Ileum	SI	Caeca	Colon	Duo	Jejunum	Ileum	SI	Caeca	Colon
without	0	0.34[a]	0.38	1.20	0.73	1.37	1.00	3.08	0.36	0.17	1.73	4.22	4.18	10.07	1.67	0.41
	0.1	0.29[b]	0.38	1.32	0.73	1.30	0.91	2.94	0.37	0.14	1.75	4.01	4.11	9.87	1.69	0.39
with	0	0.30[ab]	0.43	1.31	0.71	1.31	0.97	2.99	0.36	0.15	1.80	4.24	4.35	10.39	1.73	0.42
	0.1	0.31[ab]	0.38	1.33	0.74	1.28	0.93	2.94	0.37	0.15	1.79	4.23	4.29	10.28	1.68	0.42
SEM[2]		0.006	0.009	0.020	0.008	0.018	0.012	0.030	0.009	0.003	0.023	0.056	0.059	0.118	0.026	0.007
Main effects																
Medication																
without		0.32	0.38	1.26	0.73	1.33	0.96	3.01	0.36	0.16	1.74	4.12	4.15	9.97	1.68	0.40
with		0.30	0.40	1.32	0.73	1.30	0.95	2.97	0.37	0.15	1.79	4.23	4.32	10.33	1.71	0.42
BGase (%)																
0		0.32	0.41	1.25	0.72	1.34	0.98[a]	3.04	0.36	0.16[a]	1.76	4.23	4.27	10.23	1.70	0.42
0.1		0.30	0.38	1.32	0.74	1.29	0.92[b]	2.94	0.37	0.15[b]	1.77	4.12	4.20	10.07	1.68	0.41
Probability																
Medication		0.36	0.18	0.10	0.83	0.34	0.61	0.45	0.84	0.58	0.21	0.29	0.13	0.11	0.61	0.16
BGase		0.29	0.10	0.07	0.30	0.14	0.005	0.12	0.41	0.01	0.83	0.32	0.56	0.49	0.75	0.44
Medication × BGase		0.007	0.13	0.18	0.47	0.57	0.31	0.40	0.98	0.08	0.75	0.35	0.97	0.82	0.48	0.64

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

[1]BGase - β-glucanase; Proven - proventriculus; Duo - duodenum; SI - small intestine.

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

Table 5. 13. Effects of diet medication and β-glucanase on gastro-intestinal content and organ weights as a percentage of body weight of broiler chickens at d 28 (Experiment 1)

Medication	BGase[1] (%)	Content									Weight		
		Crop	Proven	Gizzard	Duo	Jejunum	Ileum	SI	Caeca	Colon	Liver	Spleen	Pancreas
without	0	0.28	0.03	0.93^{b}	0.09	1.03^{a}	1.17	2.29^{a}	0.30	0.19	2.40	0.10	0.24
	0.1	0.52	0.03	1.14^{b}	0.07	0.74^{b}	0.90	1.69^{b}	0.24	0.16	2.50	0.09	0.23
with	0	0.33	0.11	1.53^{a}	0.09	0.85^{ab}	1.11	2.05^{ab}	0.27	0.21	2.43	0.10	0.26
	0.1	0.45	0.03	1.31^{ab}	0.07	0.87^{ab}	1.06	2.00^{ab}	0.26	0.19	2.40	0.09	0.25
SEM[2]		0.066	0.017	0.058	0.005	0.028	0.035	0.056	0.014	0.009	0.029	0.003	0.005
Main effects													
Medication													
without		0.40	0.03	1.03	0.08	0.88	1.03	1.99	0.27	0.18	2.45	0.10	0.24
with		0.39	0.07	1.42	0.08	0.86	1.09	2.02	0.26	0.20	2.41	0.09	0.25
BGase (%)													
0		0.30^{b}	0.07	1.23	0.09^{a}	0.94	1.14^{a}	2.17	0.28	0.20	2.41	0.10	0.25
0.1		0.48^{a}	0.03	1.22	0.07^{b}	0.80	0.98^{b}	1.84	0.25	0.18	2.45	0.92	0.24
Probability													
Medication		0.92	0.22	0.0005	0.60	0.63	0.43	0.74	0.77	0.14	0.50	0.72	0.05
BGase		0.04	0.25	0.93	0.01	0.007	0.02	0.002	0.21	0.19	0.45	0.20	0.16
Medication × BGase		0.56	0.21	0.04	0.90	0.002	0.11	0.01	0.39	0.74	0.22	0.74	0.82

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

[1]BGase - β-glucanase; Proven - proventriculus; Duo - duodenum; SI - small intestine.

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

Table 5. 14. Effects of diet medication and β-glucanase on gastro-intestinal tissue weights and lengths (proportional to body weight) of broiler chickens at d 11 (Experiment 2)

Medication	BGase[1] (%)	Empty weight (%)									Length (cm/100g)					
		Crop	Proven	Gizzard	Duo	Jejunum	Ileum	SI	Caeca	Colon	Duo	Jejunum	Ileum	SI	Caeca	Colon
without	0	0.53	0.83	2.63	1.92^{a}	2.97^{a}	2.11	7.00^{a}	0.66^{a}	0.26	7.22	17.42	15.66	40.29	5.40	1.39
	0.1	0.48	0.79	2.61	1.77^{ab}	2.63^{b}	1.88	6.27^{b}	0.60^{ab}	0.22	6.90	15.16	14.97	37.02	5.24	1.36
with	0	0.46	0.87	2.69	1.51^{b}	2.40^{b}	1.74	5.65^{c}	0.50^{b}	0.25	7.13	15.45	13.56	36.14	4.62	1.40
	0.1	0.48	0.77	2.54	1.69^{ab}	2.67^{b}	1.78	6.13^{bc}	0.62^{ab}	0.25	6.11	14.64	13.75	34.49	4.99	1.34
SEM[2]		0.018	0.018	0.043	0.039	0.053	0.043	0.109	0.020	0.006	0.219	0.273	0.329	0.584	0.121	0.035
Main effects																
Medication																
without		0.50	0.81	2.62	1.84	2.80	2.00^{a}	6.64	0.63	0.24	7.06	16.29^{a}	15.31^{a}	38.65^{a}	5.32^{a}	1.37
with		0.47	0.82	2.62	1.60	2.54	1.76^{b}	5.89	0.56	0.25	6.62	15.05^{b}	13.65^{b}	35.31^{b}	4.80^{b}	1.37
BGase (%)																
0		0.49	0.85^{a}	2.66	1.72	2.69	1.93	6.33	0.58	0.25	7.17	16.43^{a}	14.61	38.21^{a}	5.01	1.39
0.1		0.48	0.78^{b}	2.58	1.73	2.65	1.83	6.20	0.61	0.24	6.50	14.90^{b}	14.36	35.76^{b}	5.12	1.35
Probability																
Medication		0.16	0.77	0.92	0.0009	0.001	0.003	<.0001	0.07	0.44	0.26	0.004	0.01	0.001	0.03	0.92
BGase		0.70	0.04	0.29	0.90	0.62	0.19	0.42	0.43	0.11	0.09	0.0007	0.69	0.01	0.65	0.41
Medication × BGase		0.15	0.42	0.41	0.02	0.0004	0.08	0.0005	0.01	0.15	0.36	0.08	0.48	0.40	0.26	0.74

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

[1]BG - β-glucanase; Proven - proventriculus; Duo - duodenum; SI - small intestine.

[2]SEM - pooled standard error of mean (n=12 birds per treatment).

Table 5. 15. Effects of diet medication and β-glucanase on gastro-intestinal content and organ weights as a percentage of body weight of broiler chickens at d 11 (Experiment 2)

Medication	BGase[1] (%)	Content									Weight		
		Crop	Proven	Gizzard	Duo	Jejunum	Ileum	SI	Caeca	Colon	Liver	Spleen	Pancreas
without	0	0.48	0.06	0.89	0.08	0.59	0.60	1.26[a]	0.08	0.06	4.05	0.13	0.57
	0.1	0.54	0.05	0.81	0.05	0.45	0.41	0.89[c]	0.11	0.04	4.74	0.11	0.50
with	0	0.29	0.11	0.99	0.05	0.53	0.51	1.08[b]	0.07	0.07	4.19	0.13	0.50
	0.1	0.37	0.06	0.73	0.04	0.45	0.44	0.93[bc]	0.07	0.05	4.48	0.12	0.49
SEM[2]		0.035	0.008	0.034	0.006	0.018	0.018	0.727	0.006	0.004	0.070	0.004	0.011
Main effects													
Medication													
without		0.51[a]	0.05	0.85	0.06	0.52	0.50	1.08	0.09[a]	0.05	4.39	0.12	0.53[a]
with		0.33[b]	0.08	0.86	0.04	0.49	0.47	1.00	0.07[b]	0.06	4.34	0.12	0.50[b]
BGase (%)													
0		0.38	0.08	0.94[a]	0.06	0.56[a]	0.55[a]	1.17	0.07	0.07[a]	4.12[b]	0.13	0.54[a]
0.1		0.46	0.05	0.77[b]	0.04	0.45[b]	0.42[b]	0.91	0.09	0.04[b]	4.61[a]	0.11	0.50[b]
Probability													
Medication		0.008	0.08	0.89	0.09	0.29	0.36	0.11	0.03	0.09	0.63	0.64	0.04
BGase		0.26	0.08	0.009	0.06	<.0001	0.0001	<.0001	0.20	0.005	0.0002	0.10	0.03
Medication × BGase		0.85	0.15	0.15	0.22	0.16	0.06	0.02	0.22	0.91	0.09	0.57	0.13

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

[1]BGase - β-glucanase; Proven - proventriculus; Duo - duodenum; SI - small intestine

[2]SEM - pooled standard error of mean (n=12 birds per treatment).

Table 5. 16. Effects of diet medication and β-glucanase on gastro-intestinal tissue weights and lengths (proportional to body weight) of broiler chickens at d 33 (Experiment 2)

Medication	BGase[1] (%)	Empty weight (%)									Length (cm/100g)					
		Crop	Proven	Gizzard	Duo	Jejunum	Ileum	SI	Caeca	Colon	Duo	Jejunum	Ileum	SI	Caeca	Colon
without	0	0.30	0.38	1.12	0.87	1.64^{a}	1.13	3.64	0.37	0.17	1.80	4.49^{a}	4.42	10.70^{a}	0.63	0.41
	0.1	0.29	0.39	1.23	0.86	1.53^{a}	1.00	3.38	0.38	0.15	1.63	3.88^{b}	3.86	9.37^{b}	0.71	0.38
with	0	0.33	0.44	1.14	0.71	1.24^{b}	0.98	2.92	0.35	0.15	1.57	3.43^{c}	3.36	8.35^{c}	0.60	0.32
	0.1	0.27	0.36	1.16	0.70	1.28^{b}	0.92	2.90	0.37	0.15	1.47	3.40^{c}	3.34	8.20^{c}	0.64	0.35
SEM[2]		0.006	0.015	0.022	0.014	0.029	0.018	0.051	0.008	0.004	0.029	0.078	0.089	0.172	0.031	0.010
Main effects																
Medication																
without		0.29	0.38	1.17	0.86^{a}	1.58	1.06^{a}	3.51^{a}	0.38	0.16^{a}	1.71^{a}	4.19	4.14^{a}	10.03	0.67	0.40^{a}
with		0.30	0.40	1.15	0.70^{b}	1.26	0.95^{b}	2.91^{b}	0.36	0.15^{b}	1.52^{b}	3.41	3.35^{b}	8.27	0.62	0.33^{b}
BGase (%)																
0		0.31^{a}	0.41	1.13	0.79	1.44	1.05^{a}	3.28^{a}	0.36	0.16	1.68^{a}	3.96	3.89^{a}	9.52	0.62	0.37
0.1		0.28^{b}	0.38	1.20	0.78	1.40	0.96^{b}	3.14^{b}	0.38	0.15	1.55^{b}	3.64	3.60^{b}	8.78	0.67	0.36
Probability																
Medication		0.80	0.57	0.62	<.0001	<.0001	0.0005	<.0001	0.36	0.01	0.0003	<.0001	<.0001	<.0001	0.15	0.0004
BGase		0.005	0.27	0.12	0.55	0.33	0.005	0.04	0.22	0.11	0.01	0.009	0.04	0.004	0.11	0.88
Medication × BGase		0.12	0.20	0.31	0.83	0.05	0.28	0.10	0.88	0.15	0.47	0.01	0.06	0.02	0.68	0.09

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

[1]BGase - β-glucanase; Proven - proventriculus; Duo - duodenum; SI - small intestine.

[2]SEM - pooled standard error of mean (n=18 birds per treatment).

Table 5. 17. Effects of diet medication and β-glucanase on gastro-intestinal content and organ weights as a percentage of body weight of broiler chickens at d 33 (Experiment 2)

Medication	BGase[1] (%)	Content									Weight		
		Crop	Proven	Gizzard	Duo	Jejunum	Ileum	SI	Caeca	Colon	Liver	Spleen	Pancreas
without	0	1.54	0.11	1.18	0.12	1.31[a]	1.49[a]	2.91[a]	0.27	0.23	3.16[a]	0.12	0.27
	0.1	1.44	0.06	1.33	0.09	0.86[b]	0.97[b]	1.91[b]	0.32	0.14	2.88[b]	0.12	0.27
with	0	1.46	0.34	1.56	0.08	1.03[b]	1.12[b]	2.21[b]	0.25	0.17	2.57[c]	0.12	0.26
	0.1	1.11	0.07	1.24	0.07	0.95[b]	0.91[b]	1.92[b]	0.27	0.17	2.58[c]	0.12	0.26
SEM[2]		0.096	0.043	0.060	0.006	0.039	0.050	0.084	0.015	0.011	0.040	0.004	0.005
Main effects													
Medication													
without		1.49	0.09	1.26	0.10[a]	1.08	1.23	2.41	0.29	0.18	3.02	0.12	0.27
with		1.28	0.20	1.40	0.07[b]	0.99	1.02	2.07	0.26	0.17	2.57	0.12	0.26
BGase (%)													
0		1.50	0.23	1.37	0.10[a]	1.17	1.31	2.56	0.26	0.20[a]	2.86	0.12	0.26
0.1		1.27	0.06	1.29	0.08[b]	0.90	0.94	1.91	0.29	0.15[b]	2.73	0.12	0.26
Probability													
Medication		0.28	0.16	0.22	0.006	0.15	0.009	0.01	0.21	0.61	<.0001	0.54	0.13
BGase		0.24	0.06	0.46	0.02	0.0002	<.0001	<.0001	0.20	0.03	0.01	0.93	0.81
Medication × BGase		0.52	0.19	0.06	0.37	0.006	0.04	0.007	0.52	0.06	0.01	0.93	0.90

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

5.4.7 Performance parameters

Interactions between medication and BGase were significant or nearly significant for BWG and FI from 0-7 d, 7-14 d (P = 0.06) and 0-28 d (P = 0.06-0.07), and F:G from 0-7 d (Table 5.18) in Experiment 1. Body weight gain and FI followed a similar response to treatments. In birds fed diets without medication, the addition of BGase tended to reduce gain or feed consumption, however in those fed diets with medication, enzyme either did not affect or increased these response criteria. For the 0-7 d F:G ratio interaction, enzyme decreased and increased feed efficiency in unmedicated diets and medicated diets, respectively.

In Experiment 2, interactions between main effects were significant for BWG for all time periods (Table 5.19), but the nature of the response changed with age. From 0-11 d, medication increased gain, while enzyme did not affect gain in birds fed diets without medication and tended to increase gain in the medicated diet. Weight gain from 11 to 22 d was increased by enzyme regardless of diet medication. From 22-32 d, enzyme increased gain in the non-medicated diets, but had no effect when diets contain medication. Overall weight gain (0-32 d) was increased by enzyme use, regardless of diet medication.

Medication and enzyme use increased FI from 0-11 d, and medication similarly increased FI from 11-22 d. Interactions between medication and enzyme were significant from 22-32 d and approached significance (P = 0.06) for the overall experiment. In both cases, the use of dietary BGase tended to decrease FI when the medication was not fed and increase FI when it was.

Interactions were found between medication and BGase for F:G in all time periods. Medication increased feed efficiency throughout the trial, but as was the case for BWG, the nature of the interaction with enzyme use changed with bird age. During the 0-11 d period, F:G increased with enzyme use when birds were fed non-medicated diets, but had no effect when the medication was used. For the remainder of the time periods, including the total trial, enzyme decreased F:G in birds fed non-medicated diets, but did not affect in broilers consumed medicated diets.

Table 5. 18. Effects of diet medication and β-glucanase on performance parameters of broiler chickens (Experiment 1)

Medication	β-glucanase (%)	BWG[1] (kg)					FI (kg)					F:G				
		d 0-7	d 7-14	d 14-21	d 21-28	d 0-28	d 0-7	d 7-14	d 14-21	d 21-28	d 0-28	d 0-7	d 7-14	d 14-21	d 21-28	d 0-28
without	0	143[a]	303	507	699	1650	167[a]	421	729	1055	2371	1.17[b]	1.39	1.44	1.53	1.45
	0.1	126[c]	296	498	656	1575	157[b]	399	705	1004	2265	1.26[a]	1.35	1.42	1.54	1.44
with	0	130[bc]	284	492	668	1573	160[ab]	387	706	1000	2251	1.23[a]	1.36	1.44	1.50	1.43
	0.1	135[ab]	301	494	677	1607	160[ab]	409	695	1012	2275	1.19[b]	1.36	1.41	1.50	1.42
SEM[2]		1.562	2.966	4.564	10.050	14.222	1.172	4.887	5.856	11.406	18.375	0.008	0.011	0.009	0.014	0.007
Main effects																
Medication																
Without		134	299	503	678	1612	162	410	717	1030	2318	1.21	1.37	1.43	1.53	1.45
With		132	292	493	673	1591	160	398	700	1006	2263	1.21	1.36	1.42	1.50	1.43
β-glucanase (%)																
0		136	293	500	684	1612	163	404	717	1027	2311	1.20	1.38	1.44	1.52	1.44
0.1		130	298	496	666	1591	159	404	700	1008	2270	1.22	1.35	1.41	1.52	1.43
Probability																
Medication		0.36	0.21	0.32	0.79	0.43	0.35	0.17	0.15	0.29	0.12	0.70	0.55	0.69	0.21	0.12
β-glucanase		0.01	0.38	0.71	0.36	0.45	0.04	0.99	0.14	0.39	0.25	0.06	0.30	0.20	0.96	0.26
Medication × β-glucanase		<.0001	0.06	0.54	0.17	0.06	0.02	0.06	0.55	0.17	0.07	<.0001	0.44	0.85	0.90	0.85

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

[1]BWG - body weight gain; FI - feed intake; F:G - feed to gain ratio.

[2]SEM - pooled standard error of mean (n=10 cages per treatment).

Table 5. 19. Effects of diet medication and β-glucanase on performance parameters of broiler chickens challenged for coccidiosis (Experiment 2)

Medication	BGase[1] (%)	BWG (kg)				FI (kg)				F:G			
		d 0-11	d 11-22	d 22-32	d 0-32	d 0-11	d 11-22	d 22-32	d 0-32	d 0-11	d 11-22	d 22-32	d 0-32
without	0	0.24^{b}	0.562^{d}	0.788^{c}	1.594^{d}	0.328	0.979	1.540^{bc}	2.846	1.321^{b}	1.617^{a}	1.939^{a}	1.721^{a}
	0.1	0.24^{b}	0.622^{c}	0.881^{b}	1.740^{c}	0.331	0.982	1.499^{c}	2.813	1.372^{a}	1.471^{b}	1.688^{b}	1.561^{b}
with	0	0.26^{a}	0.675^{b}	0.963^{a}	1.900^{b}	0.331	1.049	1.581^{ab}	2.961	1.242^{c}	1.429^{b}	1.627^{c}	1.497^{c}
	0.1	0.27^{a}	0.702^{a}	0.981^{a}	1.954^{a}	0.339	1.071	1.588^{a}	2.998	1.236^{c}	1.423^{b}	1.593^{c}	1.479^{c}
SEM[2]		0.002	0.640	0.904	0.025	0.002	0.008	0.009	0.017	0.011	0.015	0.024	0.017
Main effects													
Medication													
without		0.24	0.591	0.835	1.667	0.329^{b}	0.981^{b}	1.520	2.829^{b}	1.347	1.544	1.813	1.641
with		0.27	0.689	0.972	1.927	0.335^{a}	1.060^{a}	1.584	2.905^{a}	1.239	1.426	1.610	1.488
BGase (%)													
0		0.25	0.618	0.876	1.747	0.329^{b}	1.014	1.560	2.904	1.282	1.523	1.783	1.609
0.1		0.25	0.662	0.931	1.847	0.335^{a}	1.027	1.544	2.905	1.304	1.447	1.641	1.520
Probability													
Medication		<.0001	<.0001	<.0001	<.0001	0.01	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001
BGase		0.77	<.0001	<.0001	<.0001	0.01	0.18	0.14	0.92	0.01	<.0001	<.0001	<.0001
Medication × BGase		0.006	0.02	0.001	0.002	0.29	0.33	0.04	0.06	0.001	<.0001	<.0001	<.0001

[a-c]Means within a main effect or interaction not sharing a common superscript are significantly different ($P \leq 0.05$).
[1]BGase - β-glucanase; BWG - body weight gain; FI - feed intake; F:G - feed to gain ratio.
[2]SEM - pooled standard error of mean (n=9 pens per treatment).

5.5 Discussion

With minor exceptions, all three MW parameters were lower with the enzyme use, which confirms exogenous BGase mediated depolymerization of HB β-glucan in broiler chickens. In addition, the reduction of MW-10% with BGase in both experiments further supports β-glucan depolymerization since it demonstrates the increased proportion of small MW soluble β-glucan in ileal digesta of broiler chickens. Overall, the response for Mp was similar in both experiments, which indicates β-glucan depolymerization is independent of the disease status and the age of the animal. However, the BGase and medication effects on Mw were different from Mp in Experiment 2 at both ages; in contrast the two responses were similar in Experiment 1. The difference between Mp and Mw, and the less consistent reduction of Mw in Experiment 2 might be due to the bimodal size distribution with increased amounts of high MW β-glucan (a separate larger peak) found when diets contained BGase. The reason for the high molecular weight β-glucan is unknown but could relate to aggregation of smaller weight β-glucan molecules (Gaborieau and Castignolles, 2011; Holtekjolen et al., 2014) or enzyme-mediated release of higher MW, insoluble β-glucan that had not yet been depolymerized. The bimodal curve found in this study has also been noted in Chapter 4. The reduction of β-glucan MW and increased proportion of small MW soluble β-glucan is important to determine whether it affects performance and digestive tract characteristics through increased carbohydrate fermentation in broilers. Further, small MW β-glucan proportion might be the most important assessment in chickens for the current research since chicken microbiota preferred small molecular sugars and peptides over the complex polysaccharides and proteins in a study that investigated utilization of nutrients by chicken and human fecal microbes using an *in vitro* assay (Lei et al., 2012).

The MW values were numerically lower at d 33 compared to d 11 in Experiment 2, which might be associated with an age-related adaptation of gut microbiota to utilize fibre more effectively (Bautil et al., 2019). Further, MW parameters were lower in Experiment 1 compared to both ages in Experiment 2. Although the experiments cannot be compared statistically, it does draw attention to experimental variation. The analyses of samples were completed at three different times, but the probability that analytical error accounted for the variation is unlikely because the determination of β-glucan MW distribution using size exclusion chromatography

and Calcofluor post-column derivatization is a well-established technique in food science (Boyd et al., 2017) and all laboratory work was completed in the same lab by the senior author. A more plausible explanation for the difference relates to variation in β-glucan characteristics in the barley samples that were fed. The birds were fed diets containing CDC Fibar in both experiments however, the samples were different in the two experiments. Although they were the same cultivar, environmental conditions such as germination may have impacted β-glucan MW. High moisture content in the environment might activate endogenous enzymes in barley and degrade non-starch polysaccharides including β-glucan, which is supported by the improved nutritive value of barley with the water treatment (Fry et al., 1958). To the best of our knowledge, this, and other research in this thesis, are the first to document MW changes in soluble β-glucan as a result of exogenous BGase use in chickens fed barley diets. Beta-glucanase effect on the reduction of ileal soluble β-glucan MW in this study was in accordance to the results in Chapter 3, 4 and 6 however, no comparative data from other labs are available.

The MW parameters in the two experiments decreased with medication when there was no added BGase in the diet. This is an unexpected finding since the medication did not contain endo-β-glucanase activity. It is possibly due to the antibiotic modification of the GI microbial population (Torok et al., 2011; Singh et al., 2013; Simon et al., 2016; Xiong et al., 2018) resulting in microbiota with an increased capacity to degrade high MW β-glucan into lower MW polysaccharides and oligosaccharides. *In vitro* studies have demonstrated that strict anaerobic caecal microbiota including *Bacteroides ovatus, B. uniformis, B. capillosus, Enterococcus faecium*, *Clostridium perfringens* and *Streptococcus* strains in broiler chickens are capable of degrading mixed-linked β-glucan (Beckmann et al., 2006). The changes in the intestinal microbial populations were also reported in pigs fed barley-based diets (Pieper et al., 2008; Gorham et al., 2017), although they were not the same bacterial species in the above-mentioned *in vitro* assays. However, medication was not able to breakdown high MW β-glucan to the same extent as BGase. It demonstrates the higher efficacy of feed BGase in comparison to the BGase originated from microbiota in the chicken GIT of degrading high MW β-glucan.

Exogenous BGase depolymerizes high MW soluble β-glucan into low MW β-glucan in the ileal digesta, which leads to a reduction of ileal viscosity in broiler chickens and this is

evident in both experiments. However, medication did not affect ileal viscosity in broiler chickens, although MW was reduced with the addition of antibiotics to the broiler diets. Nevertheless, viscosity changes at 11 d appears like the molecular weight data where enzyme decreased values, but the decrease was less with medicated diets, and it is primarily due to the low viscosity in the treatments with medication when BGase was not used. In addition to the degree of polymerization of β-glucan, there might be other factors that affect intestinal viscosity including the concentration, solubility, structure and configuration of non-starch polysaccharides including β-glucan and arabinoxylan in the digesta (Boros et al., 1993; Saulnier et al., 1995; Bach Knudsen and Laerke, 2016). Further, medication might have shifted the ileal microbial population in a way that leads to increased intestinal mucus production, which can contribute to ileal viscosity (Cadogan and Choct, 2015; Morel *et al*., 2005). In addition, high amounts of NSP in the diet also increase intestinal mucus production in monogastric animals (Mälkki and Virtanen, 2001; Morel et al., 2005; Cadogan and Choct, 2015).

Overall, BGase reduced the empty weights, lengths, and weights of digesta content in GIT segments in both experiments. The reduction in size coincides with increased digestive efficiency associated with enzyme use and has been reported previously (Brenes et al., 1993a; Jørgensen et al., 1996). In addition, the reduction of GIT content weights might be associated with increased feed passage rate in the GIT (Salih et al., 1991; Almirall and Esteve-Garcia, 1994) since exogenous BGase decrease digesta viscosity, and thereby increase digestive function in the broiler chickens (Hesselman and Åman, 1986; Edney et al., 1989; Brenes et al., 1993a; Pertila et al., 2001; Ravindran et al., 2007). Further, HB mediated larger digestive tract might hold more digesta that leads to increased GIT content weights in the current study. Medication decreased the empty weights and lengths from the duodenum to colon, and the content weights of GIT segments. This is in accordance with previous research that observed a reduction of intestinal tract weights and lengths with in-feed antibiotics (Bacitracin methylene disalicylate and virginiamycin) in broiler chickens (Miles et al., 2006). The use of specific antibiotics in feed lessen the growth of pathogenic bacteria in the digestive tract of chickens through the modification of microbial diversity and relative abundance, and immune status (Lee et al., 2012; Dibner and Richards, 2015), and thereby increase nutrient digestibility. Further, diet medication

might increase nutrient digestion due to increased utilization of non-starch polysaccharides, which is supported by β-glucan MW reduction with BGase addition to the diets in the current research. The effect of medication on relative GIT size and content weights is mostly significant when the HB based diets did not contain BGase, since the enzyme also decreases GIT size by increasing nutrient digestibility in broiler chickens.

Levels of SCFA and pH in the digestive tract were used to estimate the effects of diet BGase and antibiotics on carbohydrate fermentation. Diet BGase and medication depolymerized soluble β-glucan in HB in the ileal digesta of broiler chickens, which may influence carbohydrate fermentation in the lower GIT. Ileal pH was higher with BGase use at both ages of broiler chickens in Experiment 2. Beta-glucanase mediated increase in ileal pH is contradictory to the current hypothesis of an enzyme-dependent enhancement of carbohydrate fermentation that might be expected based on a large quantity of low MW β-glucan resulting from high MW β-glucan depolymerization due to enzyme use. This might relate to the increased feed passage rate from the ileum to caeca with the reduction of soluble β-glucan MW, which permits less time for the bacterial fermentation in the ileum (Chapter 4). However, the ileal pH is contradictory to total and individual SCFA concentrations in the ileum since SCFA levels were increased by BGase at d 33 in the current study. Reduction of caecal pH with the enzyme (d 11 without medication; d 33) might indicate increased carbohydrate fermentation in the caeca, and it followed the study completed by Józefiak et al, (2005). Further, BGase increased SCFA concentrations in the caeca (d 11 without medication) in the current study which is in accordance with the caecal pH at d 11. Overall, the results suggest BGase has shifted bacterial fermentation from the ileum to caeca in broiler chickens.

The antibiotic-induced modification of the GI microbial population might affect the production of SCFA, which in turn influences the enzyme response on carbohydrate fermentation in broiler chickens. Medication affected intestinal pH in a similar manner to BGase, and similar to the findings of Engberg (2000), who found increased ileal pH and lowered caecal pH with the addition of salinomycin and Zn bacitracin to broiler diets. However, diet medication did not affect the concentrations of SCFA in the ileum, whereas it decreased total and most of the individual SCFA concentrations in the caeca in the current study, which is again contradictory to

the caecal pH. However, the reduction of caecal SCFA concentration was in accordance to the study completed by Croom et al. (2009) that used salinomycin in broiler feed. Antibiotics modulate the microbial population of GIT of chickens (Smirnov et al., 2005; Danzeisen et al., 2011; Simon et al., 2016; Xiong et al., 2018), and these microbes might not properly utilize the fermentable fibre including β-glucan in chicken GIT due to the less production of microbial-derived non-starch polysaccharidases. However, it is contradictory to the findings of the ileal β-glucan MW distribution, since medication reduced the MW, which demonstrates the presence of GI bacteria that could secrete non-starch polysaccharidases. The resulting SCFA might have been immediately utilized by microbes to produce other metabolic products and affects the measured levels of SCFA. Of note, the crop pH was higher with diet medication. The crop is colonized by BGase-secreting microbiota (Cardoso et al., 2014), and medication modifies the crop microbiota that affects carbohydrate fermentation (Rada and Marounek, 1996).

Treatment effects were evaluated on the parameters of GI histo-morphology of the ileum and observed a few main effects without any interactions. Medication increased villus height to crypt depth ratio in the ileum which is an indication of increased nutrient absorption surface (Caspary, 1992) that eventually leads to enhancement of nutrient digestion and performance of chickens. In addition, medication decreased crypt depth in the ileum. Increased crypt depth indicates high cell proliferation in the intestinal epithelial cells (Sukhotnik et al., 2009) which is an indication of injury or inflammation of intestinal mucosa, thus the mucosa enhances healing from the damage by increasing cell proliferation (Seno et al., 2009; Kuhn et al., 2014). Inflammation is a protective mechanism, although an uncontrolled and chronic inflammation may damage the affected tissues (Bamford, 1999; Ward and Lentsch, 1999). Therefore, the reduction of crypt depth is considered as a positive entity that enhances bird health. The use of specific diet medication shifts bacterial distribution in GIT of broiler chickens towards saccharolytic fermentation (Singh et al., 2013; Xiong et al., 2018), and increases SCFA production including butyrate that could increase epithelial growth in GIT of chickens (Hu and Guo, 2007), and it might be the cause for high villus height: crypt depth in the ileum. Short chain fatty acids especially butyrate has the potential to affect inflammation by regulating inflammatory cytokines (Kim et al., 2013; Singh et al., 2014; Iraporda et al., 2015; Macia et al.,

2015). However, medication did not affect total SCFA or butyrate in the ileum in the current research.

Treatment effects were significant on SCFA concentrations and intestinal pH in coccidiosis challenged broiler chickens, but not in battery-cage raised and unchallenged broiler chickens. Further, the treatment effects were largely significant on the broiler chickens aged 11 d (mostly infected with *Eimeria* spp) compared to 33 d (mostly recovered from the disease) of the coccidiosis challenged broilers. The *Eimeria* spp largely disturbs the lower GI microbial population in broilers (Hume et al., 2006; Macdonald et al., 2017) due to the epithelial damage of the intestinal mucosa, and it affects SCFA production (Leung et al., 2019). On the other hand, a precise SCFA production might not be measured in the current study due to the limitations of the digesta collection procedure. Partial absorption of SCFA to the portal circulation before a sample collection, which leads to under-estimation of the values, and caecal evacuation that affected by the time of the sample collection results in individual bird variability in results. In addition, protein fermentation affects digesta pH since some of the protein fermentation products including ammonia, indoles, phenols and biogenic amines increase pH in GIT of chickens (Apajalahti, 2005).

Performance variables were within the normal range according to Ross 308 Broiler Performance Objectives (Aviagen, 2014). The interaction of BGase and medication was significant on BWG and F:G at all the periods of the broiler production cycle in Experiment 2. Medication increased both BWG and feed efficiency of broilers considering the total production cycle, however the medication response was greater without the use of BGase since exogenous BGase positively influence growth performance in the current study. Both Zn Bacitracin and ionophore anticoccidials have been classified as growth-promoting drugs in broiler chickens due to their positive impact on body weight gain and feed efficiency (Radu et al., 1987; Elwinger et al., 1998; Engberg et al., 2000). Further, virginiamycin in the feed also improved growth performance in broiler chickens according to the study completed by Khodambashi Emami et al. (2012). Antibiotics in the diets shift the GI microbial population towards a more beneficial and diversified microbiota (Dibner and Richards, 2015). Further, a diversified and potentially beneficial caecal microbiota has been reported in broiler chickens supplemented with

virginiamycin in the feed (Dumononceaux et al., 2006). Among the beneficial changes is an increase in carbohydrate fermentation (van Der Wielen et al., 2002) including β-glucan, and positively affect GI physiology and health and, helps in improving the production performance of broiler chickens. Short chain fatty acids, especially butyric acid, produced as a result of carbohydrate fermentation, increases energy supply to intestinal epithelial cells (Pourabedin and Zhao, 2015), increases nutrient absorptive surface area by increasing villi size (Panda et al., 2009; Wu et al., 2018), and also decreases harmful pathogenic bacteria in lower GIT of chickens (van Der Wielen et al., 2002). Villi height to crypt depth ratio in the ileum increased with medication in the current study, which supports antibiotics mediated enhancement of the ileal absorptive surface area in broiler chickens. However, total, and individual SCFA concentrations in the caeca decreased with the addition of antibiotics which is contradictory to carbohydrate fermentation induced improvement of physiological and growth parameters in the current research.

The antibiotic induced changes in GI microbiota might increase nutrient digestibility that helps in improving the growth performance of broiler chickens. In-feed anticoccidials (ionophores) have been shown to decrease *Roseburia* in the chicken caeca (Danzeisen et al., 2011), a butyric acid-producing, *Clostridium* cluster XIVa bacterium that negatively affects growth performance (Duncan et al., 2002; Opapeju et al., 2009; Neyrinck et al., 2011). Accordingly, medication associated growth promotion in the broilers might have been related to the reduction of this specific organism, and it is also supported by the reduced caecal concentration of butyric acid with the use of antibiotics in the diets.

Beta-glucanase increased BWG and feed efficiency of broiler chickens after d 11, although they were lower with the use of BGase before d 11. It was in agreement with the results of broiler chickens in other experiments that entirely used diets without medication, which speculated the poor production performance in young birds (< 11 d) possibly as a result of less utilization of low MW carbohydrates due to the immature digestive system including gut microbiota of the broilers (Chapter 4). In the experiment of Chapter 4, BGase dosage of 0.01% increased broiler performance despite the age when compared to 0% BGase. However, 0.1% BGase reduced the BWG and feed efficiency in the birds aged < 11 d but increased after d 11. It

led to the application of 0.1% BGase as the dietary enzyme level in the current study. Moreover, BGase decreased the total requirement of medication in HB-based diets in terms of achieving a high production performance, as the medication response on performance variables decreased with the addition of BGase to the diets. It demonstrates the ability of BGase to partially replace diet medication in HB-based diets to feed broiler chickens. However, the effect of both medication and BGase on performance variables was not significant in the production cycle except the period of d 0-7 of broiler chickens in Experiment 1, where the birds were grown in battery cages. The environment of battery cages is relatively hygienic compared to floor pens and is consisted of less pathogenic bacterial exposure with the birds, and it might be the reason for less significant effects of medication and enzyme on production parameters in the battery cage study.

In conclusion, both feed BGase and medication can depolymerize high MW soluble β-glucan of HB into low MW β-glucan in GIT of broilers however, the response was high with BGase compared to medication. The effects of diet medication and BGase on carbohydrate fermentation is not consistent across sample collections in the two experiments according to SCFA levels and intestinal pH, although treatment effects were observed in certain instances. Exogenous BGase and medication increased the growth performance of broiler chickens. Moreover, BGase reduced the necessity of antibiotics and anticoccidials in diets to achieve a high level of production performance of broiler chickens challenged for coccidiosis.

6.0 EFFECTS OF DIET HULLESS BARLEY AND BETA-GLUCANASE LEVELS ON ILEAL DIGESTA SOLUBLE BETA-GLUCAN MOLECULAR WEIGHT AND CARBOHYDRATE FERMENTATION IN LAYING HENS

6.1 Abstract

Exogenous β-glucanase (BGase) improves nutrient digestibility and production performance in laying hens fed a barley-based diet, but the effect of enzyme dosage on these characteristics, and β-glucan depolymerization and fermentation in the gastrointestinal tract is poorly understood. Therefore, the objectives of the study were to determine the effects of hulless barley (HB) and BGase on digestive tract β-glucan depolymerization and fermentation in laying hens. A total of 108 Lohman-LSL Lite hens were housed in cages and fed two levels of HB (CDC Fibar; 0 and 73%) and graded levels of BGase (Econase GT 200 P from ABVista; 0, 0.01 and 0.1%) in a 2 × 3 factorial arrangement. Birds were fed experimental diets for 8 weeks starting at 35 weeks of age. Digestive tract samples were collected at the end of the experiment. Statistical significance was set at $P \leq 0.05$. Beta glucan peak molecular weight was lower with the 0.1 compared to both 0 and 0.01% BGase levels, whereas weight average molecular weight was lower with the 0.1 compared to 0% BGase for 73% HB. Maximum molecular weight for the smallest 10% β-glucan molecules decreased with the increasing BGase. Overall, molecular weight of β-glucan in the ileum was higher when the birds were given HB- in comparison to wheat-based diets. Ileal viscosity was higher in hens fed wheat compared to HB and lower with the 0.1 compared to the 0% BGase. Both ileal and caecal pH were not affected by the treatment. Total and major SCFA in the ileum were lower with 0.1 and sometimes with the 0.01 compared to the 0% BGase in birds fed 73% HB, but not 0% HB. Interactions between main effects were found for acetic and isobutyric acids in caeca, but trends were minor. In conclusion, exogenous BGase depolymerized high molecular weight β-glucan in HB and wheat. The effects of HB and BGase on carbohydrate fermentation were not clear, although it appears ileal SCFA production was lower with increasing levels of BGase in the diets.

Keywords: fermentation, oligosaccharides, prebiotics, viscosity, pH

6.2 Introduction

Barley is a common feed ingredient in poultry feed, but its energy content is lower compared to other cereals such as corn and wheat due to its high fibre content (Bach Knudsen, 1997; Cardoso et al., 2014). Hulless barley (HB) contains less total dietary fibre compared to conventional barley due to the absence of hull. However, it has a high amount of soluble fibre, especially soluble β-glucan, concentrated in the endosperm cell walls of the barley kernel (McNab and Smithard, 1992; Izydorczyk and Dexter, 2008). The consequence is high digesta viscosity in the small intestine of chickens (Classen et al., 1985; Salih et al., 1991), which reduces nutrient digestibility (Edney et al., 1989; Friesen et al., 1992; Fuente et al., 1995; Mathlouthi et al., 2002a; Rodríguez et al., 2012) via a number of mechanisms. However, adding exogenous BGase to barley-based diets can alleviate the negative effects by reducing digesta viscosity (Fontes et al., 2004; Ponte et al., 2008). Supplementation of BGase in barley-based diets has been reported to increase nutrient digestibility, egg production and feed efficiency, while reducing the digesta viscosity in laying hens (Jeroch, 1991; Brenes et al., 1993b; Lazaro et al., 2003a; Mathlouthi et al., 2003).

Dietary fibre including soluble arabinoxylan and β-glucan are not digested in the small intestine of chickens using endogenous enzymes but are susceptible to bacterial fermentation in the lower GIT (Józefiak et al., 2004). The caeca is the main site of fermentation in the digestive tract of chickens due to a more diverse bacterial population including anaerobic bacteria (Salantro et al., 1974), and their access is restricted to fine, less viscous, and more soluble molecules (Choct et al., 1996; Svihus et al, 2013). Barley β-glucan has a high molecular weight (MW) (Cui et al., 2000; Storsley et al., 2003, Biliaderis and Izydorczyk, 2007) and this might affect the ability of β-glucan to enter the caeca since MW of carbohydrates is positively correlated to digesta viscosity of chickens (Bedford and Classen, 1992). Therefore, it is important to study the effect of exogenous BGase on depolymerizing barley β-glucan into low MW carbohydrates, which might increase the ability to enter the caeca. It has been observed supplementation of non-starch polysaccharidases increases caecal fermentation while reducing ileal fermentation of carbohydrates including arabinoxylan in chickens, which supports the increased ability of fibre getting into caeca with the use of enzymes (Choct et al., 1996; 1999).

Further, low MW arabinoxylan in wheat increased the beneficial microbial population in chicken caeca (Courtin et al., 2008b), which might indicate the alteration of carbohydrate fermentation in chickens. However, the modification of microbial population and carbohydrate fermentation might be related to the higher entry of low MW carbohydrates into the site of fermentation, or the increased efficacy of enzyme on the low over high MW carbohydrates that have already entered into the caeca, which needs further investigation. Therefore, it is logical to investigate the effect of β-glucan, which is the main non-starch polysaccharide in HB on fermentation by depolymerizing high MW β-glucan into low MW β-glucan using a very high level of exogenous BGase. In addition, the data is not available regarding barley β-glucan MW reduction in chicken digesta, although soluble barley β-glucan MW is depolymerized up to 60 and 80% in the duodenum and ileum, respectively in pigs (Holtekjolen et al., 2014).

The effect of dietary enzyme use in barley-based diets on carbohydrate fermentation has been studied in broiler chickens (Józefiak et al., 2005; 2006), however the results have been inconsistent. Enzyme either increased or had no effect on SCFA levels in the crop and caeca and had no effect on ileal levels. Similar studies have not been performed in laying hens. The degree and the exact location of carbohydrate fermentation in the GIT of laying hens might be different from broiler chickens since laying hens are older in age and have a more mature digestive tract and complex GI microbial population (Videnska et al., 2014). Moreover, differences in dietary calcium content may influence fermentation due to its high buffering capacity. Interpretation of the effects of enzyme use in barley diets is confounded using both β-glucanase and xylanase in research studies (Brenes et al., 1993b; Mathlouthi et al., 2003a; Józefiak et al., 2005; 2006). Consequently, substrates for fermentation would include low MW carbohydrates derived from arabinoxylan as well as β-glucan (Bach Knudsen, 2014). The current study is distinct from the previous research due to the use of a purified form of BGase, which permits evaluation of the single effect of BGase and HB β-glucan on fermentation in chickens.

The impact of digestive tract fermentation has practical importance in laying hen flocks as it may influence the digestive tract microbiota and consequently bird health and food safety (Ding et al., 2018). Food safety is an important consideration in laying hen flocks since salmonellosis in humans is often associated with poultry and poultry products such as eggs (Dale

and Brown, 2013; Whiley and Ross, 2015). In addition, digestive tract diseases are usually not a problem in cage housed laying hens, but hens housed on the floor (with access to excreta) are susceptible to the same diseases seen in broiler flocks. Therefore, understanding how feed and feed additives, including exogenous enzymes, play a role in controlling infectious diseases is important.

Barley β-glucan modifies gastro-intestinal microbial colonization due to high digesta viscosity, long feed retention time (Salih et al., 1991; Almirall and Esteve-Garcia, 1994) and alteration of carbohydrate fermentation (Józefiak et al., 2005; 2010) in the digestive tract of chickens. The modification of gut microbiota leads to the invasion of pathogenic bacteria in the digestive tract of chickens (Annett et al., 2002; Timbermont et al., 2011), which further affects gut and overall health. Therefore, understanding how exogenous BGase level affects the digesta soluble MW distribution of β-glucan, and consequential carbohydrate fermentation might be beneficial in terms of improving laying hen health, which directly affects food safety and public health.

The objectives of the study were to study the effects of diet HB and BGase on soluble ileal β-glucan depolymerization and carbohydrate fermentation in laying hens. It was hypothesized BGase will depolymerize high MW β-glucan in HB and the high level of low MW β-glucan will increase carbohydrate fermentation in the digestive tract of laying hens.

6.3 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).

6.3.1 Birds and housing

A total of 108 Lohman-LSL Lite hens were used for the experiment. Day-old Lohman-LSL Lite chicks were obtained from a commercial hatchery and raised on litter floor pens at the Poultry Centre of University of Saskatchewan, SK, Canada. Pullet rearing, feeding, lighting and other environmental conditions approximated Lohman recommendations (Lohman-LSL Lite Management Guide, 2013). Pullets were transferred to Layer Specht conventional cages (60.96

cm length, 39.37 cm width and 40 cm height: 502.75 cm^2/bird) in an environmentally controlled barn at 18 weeks of age. Each cage was equipped with a lubing nipple drinker and a feed trough along the length of the cage. Barn temperature was maintained at approximately 21°C and hens were provided with 16 hours of light per day at a light intensity of 10 lux at the feeder level. Hens were fed a commercial laying hen ration that met Lohman recommendations until the start of the experiment at 35 weeks of age, after which experimental diets were fed for eight weeks. Dietary treatments were randomly assigned to individual cages (10 cages per treatment) housing three birds per cage.

6.3.2 Experimental diets

The experiment was a 2 × 3 factorial arrangement with two levels of hulless barley (CDC Fibar; β-glucan – 8.0%; 0 and 73%) and three levels of BGase (Econase GT 200 P from ABVista, Wiltshire, UK; 0, 0.01 and 0.1%) providing 0, 20,000 and 200,000 BU/kg, respectively, of enzyme activity. Diets were formulated to meet Lohman-LSL Lite specifications (Lohman-LSL Lite Management Guide, 2013), and were fed in pellet form. The pelleting temperature was 65-70°C to minimize high temperature-induced BGase inactivation. Feed and water were given *ad-libitum* before the trial start and through-out the study period. Dietary composition and calculated nutrient levels are shown in Table 6.1. The estimated enzyme activity values and the analyzed BGase activity values were similar, indicating BGase was not lost during feed processing. Further, the activity of xylanase was non-detectable.

Table 6. 1. Ingredients and calculated nutrient levels of experimental diets

Ingredient	Quantity (%)
Cereal grain (wheat or hulless barley)[1]	72.56
Soybean meal	14.13
Canola oil	1.79
Mono-dicalcium phosphate	1.09
Limestone	9.38
Sodium chloride	0.31
Vitamin-mineral premix[2]	0.50
Choline chloride	0.10
Rovimix HYD 62.5	0.04
DL-Methionine	0.09
L-Lysine HCl	0.01
Nutrient, calculated	
AME (kcal/kg)	2800
Crude protein	16.04
Crude fat	3.34
Calcium	3.73
Chloride	0.28
Non-phytate phosphorous	0.40
Potassium	0.40
Sodium	0.61
Linoleic acid	1.18
Digestible arginine	0.82
Digestible Isoleucine	0.51
Digestible leucine	1.00
Digestible lysine	0.61
Digestible methionine	0.30
Digestible methionine and cysteine	0.56
Digestible threonine	0.46
Digestible tryptophan	0.18
Digestible valine	0.62

[1]Wheat - total dietary fibre (TDF) 16.1, insoluble dietary fibre (IDF) 13.6, soluble dietary fibre (SDF) 2.5, total β-glucan 0.75; hulless barley - TDF 29.1, IDF 19.3, SDF 9.8, total β-glucan 8.03 - (% DM basis).

[2]Vitamin-mineral premix provided the following per kilogram of complete diet: vitamin A (retinyl acetate + retinyl palmitate), 8000 IU; vitamin D_3, 3000 IU; vitamin E (dl-α-tocopheryl acetate), 25 IU; menadione, 1.5 mg; thiamine, 1.5 mg; riboflavin, 5.0 mg; niacin, 30 mg; pyridoxine, 1.5 mg; vitamin B_{12}, 0.012 mg; pantothenic acid, 8.0 mg; folic acid, 0.5 mg; biotin, 0.06 mg; copper, 5 mg; iron, 80 mg; manganese, 80 mg; iodine, 0.8 mg; zinc, 80 mg; selenium, 0.3 mg; calcium carbonate, 500 mg; ethoxyquin, 0.625 mg; wheat middlings, 3822.79 mg.

6.3.3 Data collection and sample collection

The total number of eggs and the presence of cracked, broken, double-yolked, soft-shelled and abnormal eggs (eg- misshapen eggs) recorded daily on a cage basis from Monday to Friday, and total numbers were mathematically calculated. Hen-day egg production (HDP) was calculated by dividing the total number of eggs per time period by the number of hen-days. Hen-housed production (HHP) was calculated by dividing the total number of eggs per time period by the number of hens housed at the beginning of the experiment. Individual body weights were measured at the start and end of the experiment. Feed intake was measured every four weeks on a cage basis. Mortality was recorded daily during the experiment period.

At the end of the experiment (43 weeks of age), all the hens were euthanized by intravenous administration of T61 containing Embutramide, Mebezonium iodide and tetracaine hydrochloride (Merck Animal Health, Kirkland, Quebec, Canada) into the brachial vein. Individual bird weights were recorded. Two birds per cage were used to measure *in situ* pH values in mid ileal and caecal contents using a Beckman Coulter 34 pH meter (Model PHI 34, Beckman instruments, Fullerton, CA). A portion of the ileal and caecal contents were collected into plastic centrifuge tubes separately from two birds and stored at -20°C until the analysis of SCFA. The rest of the ileal content from all the birds in each cage was pooled and collected into plastic snap-cap vials. A portion of the pooled ileal content was centrifuged for 3-5 min at 14,000 rpm using a Beckman microfuge (Model E348720, Beckmann instruments, INC, Palo Alto, CA). The ileal supernatant was stored in plastic micro-centrifuge tubes at -80°C for β-glucan MW distribution analysis. The viscosity of the pooled ileal supernatant was measured using a Brookfield digital viscometer (Model LVDV-III, Brookfield Engineering Labs, INC, Stoughton, MA 02072).

6.3.4 Nutritional analysis of experimental diets

Experimental diets and grains (HB and wheat) were ground through 1 mm (CP, fat, ash, insoluble and soluble dietary fibre) and 0.5 mm (total starch, β-glucan) screen-hole sizes using a Retsch laboratory mill (Retsch ZM 200, Germany) prior to chemical analysis. Experimental diets

were analyzed for β-glucan, moisture, total starch, CP, fat, ash, insoluble dietary fibre (IDF) and soluble dietary fibre (SDF). The grains were also analyzed for IDF, SDF, β-glucan, total starch, CP, fat, minerals and moisture. Beta-glucan was analyzed (AOAC Method 995.16, AACC Method 32-23 and ICC Standard Method No. 168) using a Megazyme analysis kit (Mixed-linkage beta-glucan assay procedure/McCleary method, Megazyme International Ireland Ltd., Bray Business Park, Bray, Co. Wicklow, Ireland). Moisture was analyzed using method 930.15 of AOAC. Total starch analysis was completed based on AOAC method 996.11 and AACC method 76-13.01 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, USA) and 6.25 was used as the N to CP conversion factor. Fat content was analyzed by ethyl ether extraction using Goldfish Extraction Apparatus (Labconco model 35001; Labconco, Kansas, MO, USA) following the AOAC method 920.39. Ash content was determined according to AOAC method 942.05 using a muffle oven (Model Lindberg/Blue BF51842C, Asheville, NC 28804, USA). Insoluble and soluble dietary fibre were analyzed using a Megazyme kit (Total dietary fibre assay procedure, Megazyme International Ireland Ltd., Bray Business Park, Bray, Co. Wicklow, Ireland) according to the AOAC method 991.43 and AACC method 32-07.01, and total dietary fibre (TDF) was calculated by adding the values for SDF and IDF. Mineral analysis was completed by SGS Agrifood Laboratories (Guelph, ON, Canada) following methods A202, A203a and A204a. Experimental diets were analyzed for enzyme activity (both BGase and xylanase) using β-glucan and xylazyme tablets as substrates, respectively and the end-point determination of reducing sugars (for BGase activity) and release dye (for xylanase activity) (AB Vista Methods of ESC Standard Analytical Method SAM042-01 and SAM038 respectively).

6.3.5 Beta-glucan molecular weight distribution

Ileal supernatant was analyzed for β-glucan MW distribution using size exclusion chromatography followed by calcofluor post-column detection for fluorescent recognition. Ileal supernatant was boiled for 15 min to inactivate endogenous BGase activity in the sample and then centrifuged for 5 min at 10,000 rpm using a Beckman microfuge (Model E348720,

Beckmann instruments, INC, Palo Alto, CA) before analysis of the samples using HPLC. The two columns used for HPLC were a Shodex OHpak SB-806M column with OHpak SB-G guard column and a Waters Ultrahydrogel linear column. Tris buffer (0.1M; pH=8) was used as the mobile phase. Beta-glucan peak molecular weight (Mp) and weight average molecular weight (Mw) of each sample were determined using a molar mass distribution curve (Boyd et al., 2017). Peak molecular weight is defined as the MW of the most abundant proportion of β-glucan molecules. The definition of Mw is the average (considering the weight fraction of each molecule type) of the MW of all β-glucan molecules. The maximum MW for the smallest 10% β-glucan molecules was also analyzed based on the molar mass distribution curve.

6.3.6 Short chain fatty acids analysis

Short chain fatty acids were analyzed in triplicate according to the procedure described by Zhao et al. (2006) with minor changes. The internal standard for the analysis was made up of 20 ml of 25% phosphoric acid, 300 µl of isocaproic acid and deionized water. Three hundred microliters of acetic acid, 200 µl of propionic acid, 100 µl of butyric acid and 50 µl of isobutyric, isovaleric, valeric caproic and lactic acids were used to make the standard solution. Digesta samples were thawed and mixed with 25% phosphoric acid at a 1:1 ratio and kept at room temperature for 10 min with occasional shaking. The mixture was then centrifuged at 12,000 rpm for 10 min. The supernatant (1 ml) was mixed with 1 ml of the internal standard and centrifuged at 16,000 rpm for 10 min. The sample was filtered using a 0.45-micron nylon filter, and the filtrate was filled into a GC autosampler vial and injected into the Zebron Capillary Gas chromatography column (length: 30m, internal diameter: 0.25 mm; film thickness: 0.25 µm; Zebron^{TM}ZB-FFAP, Phenomenex, Torrance, CA). The SCFA analysis was completed using a Thermo Scientific Gas chromatography system (Model Trace 1310, Milan, Italy).

6.3.7 Statistical analysis

The experiment was a complete randomized design. The cage was considered the experimental unit. There were 6 replications per treatment. Data were analyzed using SAS 9.4 Proc mixed model and the egg data were log-transformed before the analysis when necessary. The level of significance was set at $P \leq 0.05$. Mean separation was completed using Tukey-Kramer test.

6.4 Results

6.4.1 Ingredient nutrient composition

Hulless barley was consisted of 29.1% TDF, 19.3% IDF, 9.8% SDF and 8.03% total β-glucan. The values for TDF, IDF, SDF and total β-glucan in wheat were 16.1, 13.6, 2.5 and 0.75%, respectively. In HB, total starch, CP, fat, and ash were determined as 52.1, 16.6, 2.4 and 1.8%, respectively, whereas the same parameters were 65.6, 15.4, 1.4 and 1.8%, respectively, in wheat.

6.4.2 Beta-glucan molecular weight distribution

The interactions between the main effects were significant for Mp and Mw of soluble β-glucan in the ileal digesta of laying hens (Table 6.2). Beta-glucanase did not affect Mp and Mw when the birds were given a wheat-based diet. However, Mp was lower for the 0.1 compared to 0 and 0.01% BGase levels when the hens were fed 73% HB. In addition, Mw was lower for the 0.1 compared to the 0% BGase level in birds fed 73% HB diets; the average Mw for the 0.01% BGase treatment was intermediate. Further, Mw was higher with the 73 than the 0% HB when the birds were not fed BGase. The interaction was not found for the β-glucan MW-10% of ileal digesta. However, it was higher for the HB than wheat diets, and decreased with the increasing BGase. The effect of BGase on MW change in HB diets is shown in Figures 6.1A and 6.1B.

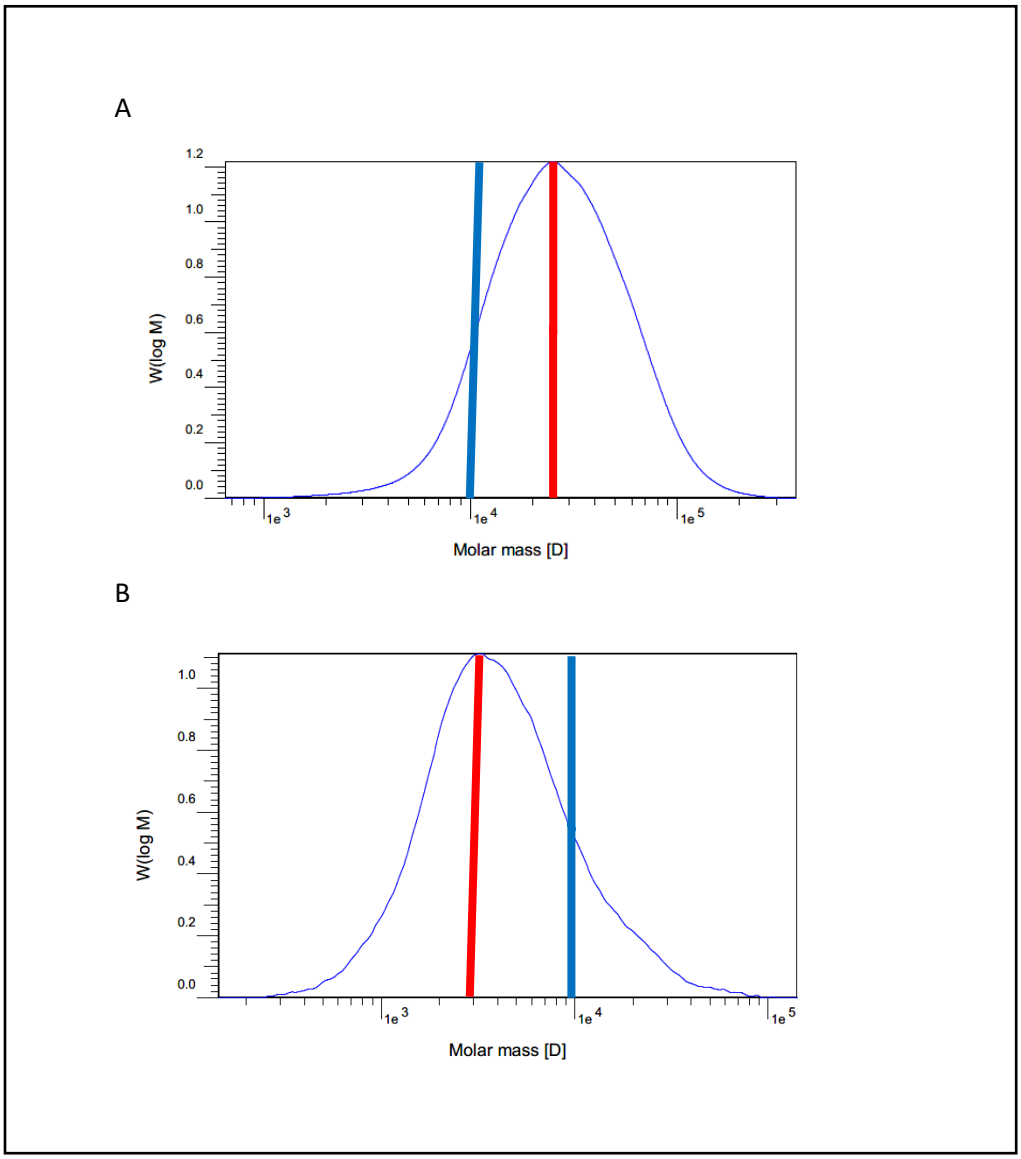

Figure 6. 1. Beta-glucan molecular weight distribution in soluble ileal digesta from laying hens fed 73% hulless barley diets. Blue lines denote point 1e4 on the x-axis and red lines indicate the Mp of the distribution curve. A. 0% β-glucanase; B. 0.1% βlucanase

Table 6. 2. Effects of hulless barley and β-glucanase on soluble β-glucan molecular weight in ileal content of laying hens at 43 weeks of age

Hulless barley (%)	β-glucanase (%)	Molecular weight (g/mol)		
		Mp[1]	Mw	MW-10%
0	0	14210[ab]	18174[b]	5365
	0.01	12521[ab]	16543[b]	4113
	0.1	11019[b]	19079[ab]	1288
73	0	19393[a]	27431[a]	7833
	0.01	19864[a]	20205[ab]	4502
	0.1	8184[b]	11509[b]	1759
SEM[2]		1017.2	1122.8	442.3
Main effects				
Hulless barley (%)				
0		12583	17932	3589[b]
73		15814	19715	4698[a]
β-glucanase (%)				
0		16802	22802	6599[a]
0.01		16192	18374	4308[b]
0.1		9601	15294	1524[c]
Probability				
Hulless barley		0.04	0.30	0.04
β-glucanase		0.001	0.004	<.0001
Hulless barley × β-glucanase		0.03	0.001	0.19

[a-b]Means within a main effect or interaction not sharing a common superscript are significantly different ($P \leq 0.05$).
[1]Mp - peak MW; Mw - weight average MW; MW-10% - The maximum MW for the smallest 10% molecules.
[2]SEM - pooled standard error of mean (n=6 cages per treatment).

6.4.3 Viscosity

Laying hen ileal soluble digesta viscosity was not affected by the interaction between HB and BGase levels (Table 6.3). However, the main effects were significant with viscosity levels lower for the 73 than the 0% HB level and viscosity decreasing with increasing BGase level; viscosity was lower for the 0.1 than 0% treatment, and the 0.01% level was intermediate and not different than the lower or higher levels.

Table 6. 3. Effects of hulless barley and β-glucanase on ileal viscosity of laying hens at 43 weeks of age

Hulless barley (%)	β-glucanase (%)	Viscosity (cP)
	0	6.90
0	0.01	6.74
	0.1	5.77
	0	5.23
73	0.01	4.07
	0.1	3.36
SEM[1]		0.268
Main effects		
Hulless barley (%)		
0		6.47[a]
73		4.22[b]
β-glucanase (%)		
0		6.06[a]
0.01		5.41[ab]
0.1		4.56[b]
Probability		
Hulless barley		<.0001
β-glucanase		0.002
Hulless barley × β-glucanase		0.42

[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 (n=6 cages per treatment).

6.4.4 Short chain fatty acids and intestinal pH

Interactions between HB and BGase were found for the ileal concentrations of total and major SCFA (acetic, propionic, butyric, and lactic acids) in laying hens (Table 6.4). Beta-glucanase level did not affect concentrations of SCFA when the hens were fed 0% HB. However, SCFA values decreased incrementally with increasing BGase levels when the birds were fed 73% HB-based diets. Statistically, 0.1% BGase levels were either lower than the 0% BGase treatment (propionic and butyric acids), or both 0 and 0.01% BGase levels (total, acetic and lactic acids). The interaction was also significant for the concentration of caproic acid, but

differences were minor and did not follow obvious trends. Isobutyric, valeric and isovaleric acid levels decreased with increasing BGase in the diets.

Main effect interactions were found for the molar percentages of acetic, propionic and caproic acids. Acetic and propionic acid percentages were higher with the 0.1% BGase compared to 0% BGase at the 0% HB. In addition, propionic acid percentage increased with the increasing BGase at the 73% HB. The interaction for caproic acid concentration again did not follow biological trends. Proportional levels of valeric, isovaleric and lactic acids were affected by BGase with a tendency for valeric and isovaleric values to be lowest, and lactic acid values to be highest for 0.01% BGase.

Total caecal SCFA levels were not affected by HB, BGase or their interaction (Table 6.5). Interactions between HB and BGase were found for caecal acetic and isobutyric acid concentrations. There was no effect of BGase on these acid concentrations for the 0% HB treatments. However, the concentration of acetic acid was lower with the 0.1% BGase compared to the 0% BGase in hens fed 73% HB. Further, isobutyric acid concentration was lower with the 0 compared to 0.01 and 0.1% BGase levels when the birds were given 73% HB-based diets. The propionic acid concentration was higher for the 0% BGase fed birds compared to those fed 0.01 and 0.1% BGase levels. Isovaleric and caproic acid concentrations were higher when birds were fed 73% HB than those fed 0% HB. The valeric acid concentration was lower for the 0% BGase compared to the diets containing 0.01 and 0.1% BGase. Furthermore, caproic acid concentration was low with the 0% BGase compared to the 0.01% BGase.

The molar percentages of caecal acetic, isobutyric and valeric acid concentrations were affected by the interactions between HB and BGase treatments (Table 6.5). Acetic acid was higher for 0 compared to 0.01 and 0.1% BGase levels when HB was fed, but the enzyme did not affect proportional values when wheat was the diet grain. Isobutyric acid was also not affected by the enzyme in hens fed wheat-based diets, but in contrast, was lower for the 0 compared to 0.01 and 0.1% BGase in HB diets. The valeric acid percentage was higher for 0.01 than 0.1% BGase at the 0% HB, whereas an enzyme effect was not found for hens fed 73% HB. The propionic acid percentage was higher for 0 than 73% HB. Further, the propionic acid percentage was affected by BGase with the ranking from high to low being 0, 0.1 and 0.01%. The

percentage of isovaleric acid was lower for 0 than 0.01 and 0.1% BGase. The caproic acid percentage was higher for 73% HB fed hens in comparison to those consuming 0% HB diets, and it was lower for 0 than 0.01% BGase.

Ileal and caecal pH values were not affected by HB, BGase or their interaction (Table 6.6).

Table 6. 4. Effects of hulless barley and β-glucanase on ileal short chain fatty acids of laying hens at 43 weeks of age

HB[1]	BGase	SCFA µmol/g of wet ileal content									Molar percentage of total SCFA							
(%)	(%)	Total	Ace	Pro	But	Isob	Val	Isov	Cap	Lac	Ace	Pro	But	Isob	Val	Isov	Cap	Lac
	0	121.0^{b}	44.6^{b}	17.0^{a}	8.2^{ab}	2.5	2.5	2.5	1.0^{a}	42.3^{b}	36.9^{b}	14.0^{b}	6.8	6.8	2.0	2.0	0.9^{a}	35.0
0	0.01	120.9^{b}	45.9^{b}	17.0^{a}	8.0^{abc}	0.9	2.0	1.9	0.9^{ab}	44.0^{ab}	38.0^{ab}	14.1^{b}	6.6	6.6	1.5	1.7	0.8^{a}	36.4
	0.1	114.5^{bc}	44.3^{bc}	16.9^{a}	7.5^{abc}	0.0	1.6	1.8	0.9^{ab}	41.2^{bc}	38.7^{a}	14.7^{a}	6.6	6.6	1.6	1.4	0.8^{a}	35.9
	0	138.7^{a}	54.1^{a}	18.1^{a}	8.8^{a}	2.7	2.6	2.7	1.1^{a}	48.3^{a}	39.0^{a}	13.1^{c}	6.3	6.3	1.9	1.9	0.8^{a}	34.7
73	0.01	118.1^{b}	45.9^{b}	16.5^{ab}	7.1^{bc}	0.8	1.4	1.4	0.6^{b}	44.0^{ab}	39.0^{a}	14.0^{b}	6.0	6.0	1.1	1.1	0.5^{b}	37.2
	0.1	101.8^{c}	39.1^{c}	14.8^{b}	6.6^{c}	0.0	1.8	2.2	0.9^{ab}	36.2^{c}	38.4^{ab}	14.5^{a}	6.5	6.5	2.1	1.7	0.9^{a}	35.5
SEM[2]		1.89	0.73	0.21	0.15	0.15	0.11	0.11	0.04	0.68	0.17	0.07	0.12	0.09	0.09	0.09	0.03	0.17
Main effects																		
HB (%)																		
0		118.8	44.9	16.9	7.9	1.1	2.0	2.1	1.0	42.5	37.8	14.3	6.6	6.6	1.7	1.7	0.8	35.8
73		119.5	46.3	16.5	7.5	1.2	1.9	2.1	0.9	42.8	38.8	13.9	6.3	6.3	1.7	1.6	0.7	35.8
BGase (%)																		
0		129.8	49.3	17.6	8.5	2.6^{a}	2.6^{a}	2.6^{a}	1.1	45.3	37.9	13.5	6.5	6.5	2.0^{a}	2.0^{a}	0.8	34.9^{c}
0.01		119.5	45.9	16.7	7.5	0.9^{b}	1.7^{b}	1.6^{b}	0.8	44.0	38.5	14.0	6.3	6.3	1.3^{b}	1.4^{b}	0.6	36.8^{a}
0.1		108.1	41.7	15.8	7.1	0.0^{c}	1.7^{b}	2.0^{ab}	0.9	38.7	38.5	14.6	6.5	6.5	1.9^{a}	1.6^{ab}	0.8	35.7^{b}
Probability																		
HB		0.80	0.18	0.21	0.14	0.79	0.72	0.95	0.19	0.78	0.003	<.0001	0.06	0.06	0.95	0.54	0.13	0.85
BGase		<.0001	<.0001	0.001	0.001	<.0001	0.002	0.002	0.003	<.0001	0.21	<.0001	0.55	0.55	0.004	0.04	0.004	<.0001
HB × BGase		0.0002	<.0001	0.002	0.05	0.89	0.16	0.26	0.04	0.001	0.008	0.0003	0.54	0.54	0.08	0.14	0.01	0.18

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

[1]HB - hulless barley; BGase - β-glucanase; SCFA - short chain fatty acids; Ace - Acetic acid; Pro - Propionic acid; But - Butyric acid; Isob - Isobutyric acid; Val - Valeric acid; Isov - Isovaleric acid; Cap - Caproic acid.

[2]SEM - pooled standard error of mean (n=12 birds per treatment).

Table 6. 5. Effects of hulless barley and β-glucanase on caecal short chain fatty acids of laying hens at 43 weeks

HB[1]	BGase	SCFA µmol/g of wet caecal content								Molar percentage of total SCFA						
(%)	(%)	Total	Ace	Pro	But	Isob	Val	Isov	Cap	Ace	Pro	But	Isob	Val	Isov	Cap
	0	322.3	194.3^{ab}	71.6	33.1	9.9^{a}	3.2	8.0	1.8	60.3^{ab}	22.2	10.2	3.0^{a}	2.4^{ab}	0.9	0.5
0	0.01	317.4	185.3^{ab}	66.5	31.6	9.9^{a}	9.7	9.8	4.2	58.3^{b}	20.9	9.9	3.1^{a}	3.1^{a}	3.0	1.3
	0.1	323.1	194.9^{ab}	69.7	32.4	10.4^{a}	6.9	5.9	2.6	60.3^{ab}	21.6	10.0	3.2^{a}	1.8^{b}	2.1	0.7
	0	342.9	209.2^{a}	74.1	34.4	5.8^{b}	5.5	10.1	3.6	61.1^{a}	21.6	10.0	1.5^{b}	2.9^{ab}	1.5	1.0
73	0.01	330.9	193.7^{ab}	68.9	33.4	10.2^{a}	10.1	10.1	4.3	58.4^{b}	20.8	10.0	3.1^{a}	3.0^{a}	3.0	1.3
	0.1	304.8	178.2^{b}	64.6	30.2	9.6^{a}	8.7	9.5	3.7	58.4^{b}	21.2	9.9	3.1^{a}	3.1^{a}	2.8	1.2
SEM[2]		4.25	2.59	0.86	0.44	0.38	0.54	0.41	0.24	0.24	0.09	0.11	0.03	0.12	0.16	0.07
Main effects																
HB (%)																
0		320.9	191.5	69.3	32.4	10.1	6.6	7.9^{b}	2.9^{b}	59.7	21.6^{a}	10.1	3.1	2.4	2.0	0.8^{b}
73		326.2	193.7	69.2	32.7	8.5	8.1	9.9^{a}	3.9^{a}	59.3	21.2^{b}	10.0	2.6	3.0	2.4	1.1^{a}
BGase (%)																
0		332.6	201.8	72.8^{a}	33.8	7.8	4.3^{b}	9.1	2.7^{b}	60.7	21.9^{a}	10.1	2.3	2.7	1.2^{b}	0.79^{b}
0.01		324.2	189.5	67.7^{b}	32.5	10.1	9.9^{a}	10.0	4.2^{a}	58.4	20.9^{c}	10.0	3.1	3.0	3.0^{a}	1.3^{a}
0.1		314.0	186.6	67.1^{b}	31.3	10.0	7.8^{a}	7.7	3.2^{ab}	59.4	21.4^{b}	9.9	3.2	2.4	2.4^{a}	1.0^{ab}
Probability																
HB		0.53	0.65	0.95	0.73	0.02	0.12	0.01	0.03	0.40	0.03	0.25	0.005	0.01	0.11	0.03
BGase		0.19	0.02	0.009	0.07	0.01	<.0001	0.07	0.01	<.0001	<.0001	0.06	0.0002	0.09	<.0001	0.008
HB × BGase		0.13	0.02	0.09	0.13	0.02	0.68	0.23	0.32	0.02	0.54	0.13	0.001	0.05	0.48	0.27

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

[1]HB - hulless barley; BGase - β-glucanase; SCFA - short chain fatty acids; Ace - Acetic acid; Pro - Propionic acid; But - Butyric acid; Isob - Isobutyric acid; Val - Valeric acid; Isov - Isovaleric acid; Cap - Caproic acid.

[2]SEM - pooled standard error of mean (n=12 birds per treatment).

Table 6. 6. Effects of hulless barley and β-glucanase on the digestive tract pH of laying hens at 43 weeks of age

Hulless barley (%)	β-glucanase (%)	pH	
		Ileum	Caeca
0	0	7.03	6.14
	0.01	7.03	5.99
	0.1	7.26	6.21
73	0	7.34	6.18
	0.01	7.19	6.55
	0.1	7.17	6.21
SEM[1]		0.050	0.069
Main effects			
Hulless barley (%)			
0		7.11	6.11
73		7.23	6.31
β-glucanase (%)			
0		7.19	6.16
0.01		7.11	6.27
0.1		7.21	6.21
Probability			
Hulless barley		0.23	0.15
β-glucanase		0.68	0.79
Hulless barley × β-glucanase		0.26	0.18

[1]SEM - pooled standard error of mean (n=12 birds per treatment).

6.4.5 Egg production and performance variables

Hen-day production and hen-housed production of laying hens were the same since no hens died during the experiment; egg production was not affected by HB or BGase. There were no significant effects of HB or BGase on feed intake, body weight gain and uniformity of laying hens. There were no treatment effects on the overall numbers of cracked, broken, double-yolked, soft-shelled and abnormal eggs (data not shown).

Table 6. 7. Effects of hulless barley and β-glucanase on hen day production, body weight and feed intake of Lohmann LSL lite hens (35-43 weeks of age)

Hulless barley (%)	β-glucanase (%)	Hen day production (%)	Daily feed intake (g)
0	0	97.7	111.2
	0.01	96.5	117.7
	0.1	95.8	117.6
73	0	98.0	113.7
	0.01	96.3	117.2
	0.1	95.4	112.7
SEM[1]		0.55	1.03
Main effects			
Hulless barley (%)			
0		96.7	115.5
73		96.6	114.5
β-glucanase (%)			
0		97.8	112.4
0.01		96.4	117.5
0.1		95.6	115.1
Probability			
Hulless barley		0.92	0.64
β-glucanase		0.27	0.14
Hulless barley × β-glucanase		0.96	0.34

[1]SEM - pooled standard error of mean (n=6 cages per treatment).

6.5 Discussion

The estimated and analyzed enzyme activity of BGase was similar, which confirms the enzyme source was added accurately and enzyme activity was not damaged during feed processing. Further, xylanase activity was non-detectable in the diets and according to our literature review this is the first time a purified BGase has been used in laying hen feed containing barley. Therefore, the current study directly demonstrates the single effect of exogenous BGase on β-glucan MW in the terminal small intestine of laying hens. Egg production and performance variables in the current research are reported to demonstrate the physiological status of the laying hens and they were not affected by dietary treatments.

Beta-glucan MW distribution of ileal digesta was analyzed to investigate if exogenous BGase depolymerizes high MW β-glucan into more low MW carbohydrates, which were hypothesized to provide increased fermentable substrate for microbes in the lower GIT of laying hens. Beta-glucan Mw and MW-10% were higher with HB than wheat, which agrees with previous research (Chapter 4 and 5; Cui et al., 2000). Further, overall β-glucan MW in the ileal digesta were approximately 10 times lower than the β-glucan MW found in the 60% HB diets without BGase (Chapter 4). This reduction may be associated with the activation of endogenous BGase in HB due to the moistening of grain when it interacts with the digestive tract secretions (Ribeiro et al., 2011). Further, the activity of microbial BGase in the upper GIT also affects the depolymerization of β-glucan in chickens (Józefiak et al., 2006; Cardoso et al., 2014). Barley cell walls may release β-glucan molecules that are associated with other non-starch polysaccharides including heteroxylans, as well as protein and phenolic acids (Burton and Fincher, 2014) with the exposure to enzymes and secretions in the chicken GIT, and contributes to the reduction of β-glucan MW. In addition, dietary BGase further reduced MW parameters, which confirms BGase mediated depolymerization of β-glucan in the ileal digesta of laying hens. Moreover, the BGase mediated reduction of MW-10% indicates an increased proportion of low MW β-glucan in ileal digesta, which might increase carbohydrate fermentation in laying hens. The production of total SCFA increased with low MW cereal β-glucan compared to high MW β-glucan according to an *in vitro* fermentation assay conducted using human faecal microbiota (Kim and White, 2010; 2011). In contrast, high MW β-glucan increased colonic SCFA compared to low MW β-glucan in pigs (Luo et al., 2019), and it might be due to the increased fermentation of low MW β-glucan in the small intestine as β-glucan is largely digested in the small intestine and only the high MW β-glucan is available for the fermentation in the colon of pigs. However, no data are available in chickens, which have a comparatively lower intestinal fermentation capacity. Of note, BGase reduced MW parameters only when hens were fed diets based on HB, but not those based on wheat. This may be due to structural differences between HB and wheat β-glucan. Beta-glucan in cereal grains predominantly yields low degrees of polymerization when exposed to lichenase. This is often referred to as the DP3: DP4 ratio and high or low DP3: DP4 ratios result in cereal β-glucan with less water-solubility and high gelling ability (Burton and Fincher, 2014). The more uniform structure leads to increased β-glucan aggregation and in turn lower solubility and

susceptibility to BGase. (Karunaratne and Classen, 2019). The addition of BGase to the HB-based diets of laying hens did not show a bimodal MW distribution curve including the high MW peak as observed in broiler chickens (Chapter 4 and 5). It might be associated with the difference in microbial community in the digestive tract of laying hens compared to broilers, which causes releasing of only a fewer insoluble β-glucan that has a high MW.

Beta-glucanase mediated ileal β-glucan MW distribution should be related to ileal viscosity since the increasing dose of BGase breaks down high MW β-glucan into lower MW carbohydrates that are less viscous. The MW of soluble carbohydrates affects the digesta viscosity of broiler chickens (Bedford and Classen, 1992) and the current research supports that conclusion in the laying hens. However, ileal viscosity was low in HB- compared to wheat-based treatments, even though the MW values of ileal digesta and diet TDF levels, including total β-glucan, were higher in HB (Mw 19715; TDF 29.1%; β-glucan 8.03%) in comparison to wheat (Mw 17932; TDF 16.1; β-glucan 0.75). This indicates other factors also determine the intestinal viscosity in chickens including the solubility, structure, and configuration of non-starch polysaccharides (Boros et al., 1993; Saulnier et al., 1995). Wheat has a higher content of arabinoxylan compared to HB, which may be a factor that increase ileal viscosity when hens were fed wheat-based diets (Choct and Annison, 1992; Kiarie et al., 2014). However, the lower ileal viscosity in birds fed HB- in comparison to wheat- based diets contrasts to grain viscosity when extracted in Na acetate buffer. The viscosity of the extracted wheat was 1.7 cP, whereas it was not measurable in HB due to very high viscosity. These data demonstrate the importance of extraction media when estimating viscosity, in this case the comparison is between Na acetate buffer and the digestive process.

The concentrations of SCFA in the ileum and caeca of laying hens were higher than the concentrations mentioned in the previous laying hen research (Johnson et al., 2008; Taylor et al., 2018) to the use of same procedure including the extraction method, and it might be associated with the difference in the age of laying hens, composition of the diets and rearing environment that eventually affects microbial population in the digestive tract of the birds. The concentrations of total SCFA and acetic, propionic, butyric, caproic and lactic acids in the ileum decreased with 0.01 and/or 0.1% BGase when the birds were fed an HB-based diet. Further, BGase decreased

ileal isobutyric, isovaleric and valeric acids in the birds given wheat and HB. These data contrast with the current hypothesis of BGase induced enhancement of carbohydrate fermentation in lower GIT of chickens. Moreover, BGase did not affect the concentrations of major SCFA in the ileum of broiler chickens given same diets (Chapter 3 and 4). However, it supports the results of Choct et al. (1999), who found that the addition of xylanase to a wheat-based diet decreased ileal SCFA compared to the control broilers fed a wheat-based diet without enzyme. It might also be associated with the increased feed passage rate as a result of the reduction of ileal viscosity by non-starch polysaccharidases (Lazaro et al., 2003b), and thereby low MW β-glucan passing rapidly into the caeca. As a result, there will be less fermentable substrate available for fermentation. If this concept is correct, carbohydrate fermentation should increase in the caeca. However, the concentrations of acetic and propionic acids in the caeca decreased with 0.1% BGase when the birds were fed HB-based diets, and isobutyric, valeric and caproic acid concentrations increased with the BGase addition. In previous research, BGase affected caecal SCFA with less clear trends, except for 0.1% BGase increased all SCFA compared to 0.01% BGase in the broilers given HB-based diets (Chapter 4). An important question is whether SCFA levels are an accurate predictor of carbohydrate fermentation. The concentration of SCFA does not only depend on the availability of fermentable substrates and microbial fermentation, but also on other factors including SCFA absorption (Mcfarlene et al., 1997). Moreover, the actual production of SCFA is impossible to obtain in poultry. The SCFA content in digesta varies with the time of sample collection since the variability of the ileal and caecal evacuation of the chickens, although the sample collections were designed to avoid treatment bias. Ileal and caecal pH of laying hens were not affected by the treatment, and therefore not related to SCFA production in the current research. However, dietary BGase consistently increased ileal pH while reducing caecal pH in broiler chickens (Chapter 3, 4 and 5), and it appears microbial fermentation has shifted from the ileum to caeca with the addition of BGase to the diets. It might be associated with the difference of the age and dietary composition of broiler chickens and laying hens that affects microbial population in the digestive tract.

In conclusion, exogenous BGase depolymerized high MW soluble HB β-glucan by the time digesta reaches the ileum, and result in a higher proportion of low MW β-glucan. In

addition, there is an enzyme dosage effect on β-glucan depolymerisation in laying hens. However, it appears BGase decreased the concentrations of major SCFA in the ileum and caeca and needs further investigation on carbohydrate fermentation and GI microbial population in laying hens to understand the mechanism of ileal soluble low MW β-glucan of HB affecting digestive tract physiological characteristics. Future research is recommended to establish an optimum dosage of BGase that might increase carbohydrate fermentation probably by further reducing MW of β-glucan in the digestive tract of laying hens.

7.0 EFFECTS OF DIETARY HULLESS BARLEY AND BETA-GLUCANASE ON *SALMONELLA* ENTERITIDIS COLONIZATION AND TRANSLOCATION IN BROILER CHICKENS

7.1 Abstract

Salmonellosis is an important public health risk and is often associated with the consumption of poultry products. Colonization of poultry by *Salmonella* Enteritidis (SE) may be affected by dietary ingredients. The objective of the study was to evaluate the effects of hulless barley (HB) and β-glucanase (BGase) on SE colonization and translocation in broiler chickens. Day-old broilers (200) were placed in battery cages and fed diets with and without high β-glucan HB (CDC Fibar; 0 and 60%) and BGase (Econase GT 200 P from ABVista; 0 and 0.1%) in a 2 × 2 factorial arrangement. Each treatment was assigned to 10 cages and there were 5 birds in each cage. *In situ* pH was measured and digesta was collected from caeca for SCFA analysis at d 14. At d 15, 4 birds from each cage were transferred to grower cages in a bio-safety level 2 facility. All the birds were orally challenged with SE LS 101 strain at d 21. Spleen and caecal samples were collected at d -2, 1, 4 and 7 of SE challenge by euthanizing 1 bird per cage on each day. Cloacal swabs were taken at -2 d of the challenge. Statistical significance was considered when $P \leq 0.05$. Caecal SCFA were not affected by the treatment. Broilers were infected with *Salmonella* pre-challenge and feeding HB decreased *Salmonella* positive cloacal swabs (%). Hulless barley decreased the birds (%) positive for *Salmonella* in the spleen at d 1 post-challenge. However, HB increased the percentage at d 4 post-challenge. There were no treatment effects on caecal *Salmonella* colonization on any collection day. Data from this research suggest feeding HB and BGase did not affect carbohydrate fermentation. Hulless barley decreased *Salmonella* counts/presence in cloacal swabs and spleen up to d 1 of SE challenge, whereas increased the counts in the spleen after d 1 of SE challenge. However, BGase did not affect *Salmonella* colonization and translocation in broiler chickens.

Keywords: Beta-glucan, prebiotics, feed enzymes, gut microbiota, zoonotic disease

7.2 Introduction

Salmonella is a gram-negative, facultatively anaerobic, non-spore forming bacilli that belong to the Enterobacteriaceae family (Cosby et al., 2015). *Salmonella enterica* is a zoonotic bacterium, and the most common *Salmonella enterica* serovars in chickens responsible for causing foodborne salmonellosis are *Salmonella* Enteritidis (SE) and *Salmonella* Typhimurium. Poultry and poultry products including raw meat and uncooked eggs are the most common sources of zoonotic *Salmonella* infection (Dunkley et al., 2009). *Salmonella* commonly results in subclinical intestinal infection in chickens, but increased mortality has been reported when chickens were infected less than two weeks old (Hogue et al., 1997).

Salmonella is one of the major food-borne pathogens which causes human gastroenteritis (Hall et al., 2005; Jonathan et al., 2017) and poultry is one of the most common origins of human salmonellosis. Therefore, Strategies to mitigate salmonellosis in chickens have been a major area of investigation (Yang et al., 2009; Huyghebaert et al., 2011). Extensive research has been conducted to investigate probiotics and prebiotics in terms of reducing *Salmonella* colonization, and most of these studies resulted in beneficial effects in broiler chickens. The colonization and translocation of *Salmonella* decreased with the addition of probiotics, specifically *Lactobacillus* spp and *Bacillus subtilis*, to the diets of poultry (Pascual et al., 1999; Higgins et al., 2008; Vila et al., 2009; Knap et al., 2011). Similarly, dietary prebiotics such as fructo-oligosaccharides and manna-oligosaccharides have been shown to reduce the colonization, translocation and susceptibility of *Salmonella* in broiler chickens and laying hens (Bailey et al., 1991; Fukata et al., 1999; Fernandez et al., 2002; Donalson et al., 2008). In addition, Eeckhaut et al. (2008) observed a reduction of SE colonization in the caeca and also a reduction of SE translocation to the spleen in broiler chickens as a result of the dietary addition of arabinoxylo-oligosaccharides.

A proposed mechanism of prebiotics is increased fermentation in the lower gastro-intestinal tract (GIT) of chickens that beneficially shifts microbial population and produces more short chain fatty acids. Short chain fatty acids are weak acids with pKa values approximating 4.8 that can lower the luminal pH of the digestive tract (von Engelhardt et al., 1998). Digestive tract pH influences microbiota since it affects the environmental conditions which are optimum for microbial growth and survival (Lauber et al., 2009). In addition, pH affects microbial

metabolism which leads to an inability of bacteria to maintain optimum pH inside the bacterial cell (Kotsyurbenko et al., 2004; Fernández-Calviño and Bååth, 2010). Arabinoxylo-oligosaccharides increased caecal bifidobacteria count (Courtin et al., 2008b), and bifidobacteria have the potential of increasing butyric acid production, which stimulates the growth of strictly anaerobic bacteria (Belenguer et al., 2006). Further, butyric acid and butyric acid esters decrease the invasion of *Salmonella* in gut epithelial cells, and colonization of the caeca and internal organs of chickens by down-regulating the expression of *Salmonella* invasion genes and improving gut barrier function (Van Immerseel et al., 2004a, b; Fernandez-Rubio et al., 2009; El-Ghany et al., 2016).

The current research focused on the effect of a potentially fermentable carbohydrate, hulless barley (HB) β-glucan, on *Salmonella* colonization in broiler chickens. Previous prebiotic research has mainly focused on the addition of the product directly into the feed of chickens. However, the current study investigates the prebiotic effect of HB β-glucan, which is a feed ingredient in the poultry diet, and by supplementing β-glucan degrading enzyme as a feed additive. Hulless barley contains of high amount of fibre, especially β-glucan, which increases digesta viscosity and feed passage time in chickens (Classen et al., 1985; Salih et al., 1991), and increases colonization of potentially pathogenic microbiota in the small intestine (Kaldhusdal and Hofshagen, 1992; Annett et al., 2002; Malthlouthi et al., 2002). However, beta-glucanase (BGase) is commonly used in the poultry feed to mitigate the negative effects associated with β-glucan when birds are fed barley-based diets. It has been demonstrated that dietary BGase depolymerizes ileal soluble high molecular weight (MW) β-glucan in HB both in broiler chickens (Chapter 3, 4 and 5) and laying hens (Chapter 6). Therefore, the resulting low MW β-glucan might have the ability to control *Salmonella* colonization and translocation probably by increasing carbohydrate fermentation in chickens. Pieper et al. (2012) observed a reduction in *Salmonella* Typhimurium translocation into the spleen due to a change of SCFA profile (increased propionic acid and decreased acetic acid molar ratios) and a shift in the microbial population in the colon when pigs were fed a HB-based diet containing a high level of β-glucan without supplementation of dietary BGase. However, the situation in chickens appears to be different and this may relate to only small, less viscous, soluble material being able to enter into

the major fermentation site (caeca) (Svihus et al., 2013). Therefore, feeding HB with the supplementation of feed BGase might be effective in delivering fermentable β-glucan into chicken caeca to minimize *Salmonella* colonization in the caeca, and translocation to the spleen. Previous research has shown the effect of BGase on the reduction of soluble β-glucan MW in the ileum of broiler chickens (Karunaratne and Classen, 2019), possibly increasing β-glucan caecal entry and fermentation. Changes in fermentation may positively affect the microbial population in chicken GIT and thereby lessen enteric disease and zoonotic colonization including SE.

The objective of the study was to investigate the effects of dietary HB and BGase on SE colonization and translocation in broiler chickens. It was hypothesized HB will increase SE colonization and translocation, whereas BGase will decrease these characteristics in broiler chickens.

7.3 Materials and methods

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

7.3.1 Birds and housing

A total of 200 0-day old male (Ross × Ross 308) broiler chickens were obtained from a commercial hatchery and housed five birds per cage in battery cages (51 cm length, 51 cm width, 46 cm height) with wire mesh floors. The room temperature was 32°C at d 0 and gradually decreased by 2.8°C per week. Day length was 23 h from day 0 to 7 and 18 h from d 8 to 15. Light intensity was a minimum of 25 lux throughout the trial. Birds were provided with *ad-libitum* feed and water throughout the experiment. Each battery cage was equipped with a front-mounted feed trough (51 cm length) and two height-adjustable nipple drinkers. Extra feed and water were supplied by supplementary feeders (d 0 to 4 of age) and ice cube trays (d 0 to 5), respectively. The dietary treatments (4) were randomly assigned to cages (equal number of replications per battery cage level for all the treatments), and there were 10 replications per treatment. Swabs were taken from the cardboard boxes used to transport the chicks from the hatchery to the

Poultry Centre. They were directly plated on Brilliant Green agar and incubated overnight at 37°C; the swabs were identified as *Salmonella*-negative. Birds were raised in the battery cage facility under strict bio-security measures. Birds were moved from these battery cages into grower cages in a bio-safety level 2 facility at d 15 for the SE oral challenge. The dietary treatments were randomly assigned to the grower cages. Four birds (the same bird groups in the previous battery cages) were placed in each cage (68.5 cm length, 63.5 cm width). Each grower cage had a front-mounted feed trough (68.5 cm length) and a trough drinker (68.5 cm length). Environmental conditions approximated the above-mentioned conditions. Feed and water were provided *ad-libitum* throughout the study period.

7.3.2 Experimental diets

Dietary treatments were applied according to a 2 × 2 factorial arrangement. Hulless barley (CDC Fibar; β-glucan – 8.0%) level and BGase (Econase GT 200 P from ABVista, Wiltshire, UK) level were the two factors used to design the diets. Hulless barley levels of 0 and 60%, and BGase levels of 0 and 0.1% (BGase activity of 0 and 200,000 BU/kg) were used to make the dietary treatments. Hulless barley was added in exchange for wheat assuming equal nutritional value. Diets were formulated according to Ross 308 broiler nutrition specifications (Aviagen, 2014) and were fed in crumble form throughout the experiment. The ingredients and calculated nutrient levels are presented in Table 7.1. The pelleting temperature was maintained at 70-75°C to prevent the inactivation of BGase due to high temperature. Measured β-glucanase activity approximated the estimated values after enzyme addition to the diets and xylanase activity was non-detectable.

Table 7. 1. Ingredients and calculated nutrient levels of the experimental diets

Ingredient	Quantity (%)
Cereal grain (wheat or hulless barley)[1]	60.00
Wheat (remaining)	5.30
Soybean meal	26.90
Canola oil	4.10
Mono-dicalcium phosphate	1.20
Limestone	1.52
Sodium chloride	0.38
Vitamin-mineral premix[2]	0.50
Choline chloride	0.10
Nutrient, calculated	
AME (kcal/kg)	3100
Crude protein	21.24
Crude fat	5.57
Calcium	0.87
Chloride	0.36
Non-phytate phosphorous	0.44
Potassium	0.83
Sodium	0.18
Digestible arginine	1.35
Digestible Isoleucine	0.81
Digestible leucine	1.47
Digestible lysine	1.15
Digestible methionine	0.54
Digestible methionine and cysteine	0.87
Digestible threonine	0.77
Digestible tryptophan	0.24
Digestible valine	0.87

[1]Wheat - total dietary fibre (TDF) 16.1, insoluble dietary fibre (IDF) 13.6, soluble dietary fibre (SDF) 2.5, total β-glucan 0.75; hulless barley - TDF 29.1, IDF 19.3, SDF 9.8, total β-glucan 8.03 - (% DM basis).

[2]Vitamin-mineral premix provided the following per kilogram of complete diet: vitamin A, 11,000 IU; vitamin D_3, 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 3773 mg.

7.3.3 Performance data collection and physiological sample collection

Bird weight and feed intake were measured on a cage basis and feed to gain ratio was calculated from these values. Mortality was recorded daily and sent to Prairie Diagnostic Services, Saskatoon, SK, Canada for necropsy. One bird per cage was euthanized by intravenous administration of T-61 (Embutramide, mebezonium iodide and tetracaine hydrochloride injectable euthanasia solution) into the brachial vein at d 14. Caecal content pH was measured using a Beckman Coulter 34 pH meter (Model PHI 34, Beckman instruments, Fullerton, CA). Caecal contents were collected into plastic centrifuge tubes and stored at -20ºC until analysis of SCFA.

7.3.4 Experimental diets analysis

The diets and ingredients (HB and wheat) were ground to 0.5- (for total starch and β-glucan analyses) and 1-mm (for CP, fat, ash and, insoluble and soluble dietary fibre analyses) screen-hole sizes using a Retsch laboratory mill (Retsch ZM 200, Germany). Samples were analyzed for β-glucan (AOAC Method 995.16, AACC Method 32-23 and ICC Standard Method No. 168) using a Megazyme analysis kit (Mixed-linkage beta-glucan assay procedure/McCleary method, Megazyme International Ireland Ltd., Bray Business Park, Bray, Co. Wicklow, Ireland) and moisture was determined according to AOAC method of 930.15. Diets were further analyzed for insoluble dietary fibre (IDF), soluble dietary fibre (SDF), total starch, CP, fat and ash. Hulless barley and wheat were also analyzed for β-glucan, insoluble dietary fibre (IDF), soluble dietary fibre (SDF), total starch, CP, fat, minerals, and moisture. Insoluble and soluble dietary fibre were analyzed using a Megazyme kit (Total dietary fibre assay procedure, Megazyme International Ireland Ltd., Bray Business Park, Bray, Co. Wicklow, Ireland) according to the AOAC method 991.43 and AACC method 32-07.01, and total dietary fibre (TDF) was calculated by adding IDF and SDF. Total starch analysis was completed using AOAC method 996.11 and AACC method 76-13.01 using a Megazyme kit (Total starch assay procedure, Amyloglucosidase/α-amylase method, Megazyme International Ireland Ltd., Bray Business Park, Bray, Co. Wicklow, Ireland). A Leco protein analyzer (Model Leco-FP-528L, Leco Corporation, St. Joseph, MA, USA) was used to analyze nitrogen and 6.25 was used as the N to CP conversion factor. Ether extraction was completed using Goldfish Extraction Apparatus

(Labconco model 35001; Labconco, Kansas, MO, USA) following AOAC method 920.39 to determine fat content. Ash content was analyzed according to AOAC method 942.05 using a muffle oven (Model Lindberg/Blue BF51842C, Asheville, NC 28804, USA). Minerals were analyzed by SGS Agrifood Laboratories (Guelph, ON, Canada) following methods A202, A203a and A204a. The diets were analyzed for BGase and xylanase activity using the methods of ESC Standard Analytical Method SAM042-01 and SAM038, respectively.

7.3.5 Short chain fatty acids analysis

Short chain fatty acids were analyzed in triplicate according to the method described by Zhao et al. (2006) with minor changes. Twenty millilitres of 25% phosphoric acid, 300 µl of isocaproic acid and deionized water were used to make the internal standard. Three hundred microliters of acetic acid, 200 µl of propionic acid, 100 µl of butyric acid and 50 µl of isobutyric, isovaleric, valeric, caproic and lactic acids were used to make the standard solution. Digesta was thawed and then mixed with 25% phosphoric acid at a ratio of 1:1. It was kept at room temperature for 10 min with occasional shaking. It was centrifuged at 12,000 rpm for 10 min. The supernatant (1 ml) was mixed with 1 ml of the internal standard and centrifuged at 16,000 rpm for 10 min. It was filtered using a nylon filter (0.45 micron), and the filtrate was added to a gas chromatograph autosampler vial and injected into the Zebron Capillary Gas Chromatography column, length: 30m; internal diameter: 0.25 mm; film thickness: 0.25 µm (Zebron^{TM}ZB-FFAP, Phenomenex, Torrance, CA). A Thermo Scientific Gas chromatography system was used for the analysis (Model Trace 1310, Milan, Italy).

7.3.6 Salmonella Enteritidis oral challenge, sample collection and processing

Salmonella Enteritidis LS101 wild type strain was obtained from VIDO-InterVac, Saskatoon, SK, Canada, and was stored at -80°C in Luria-Bertani (LB) broth and glycerol (1:1) until use. The SE LS101 was grown in an LB agar and incubated overnight at 37°C. Then an isolated colony was transferred into 5 ml of LB broth and incubated overnight at 37°C in a shaker. The bacteria-containing 800 µl of LB broth was diluted with 800 ml of normal saline to make the required volume of SE challenge and was incubated at 37°C in a shaker until the absorbance value was adjusted to an OD value of 0.7 (to ensure log-phase growth of the SE) at 600 nm (around 2 h and 40 min) with a Thermo electron Helios Gamma spectrophotometer

(Model G120845, American Laboratory Trading, Inc, East Lyme, CT), which approximately resulted in 1×10^9 colony forming units (CFU)/ml. The bacterial suspension was centrifuged at 7000 rpm for 10 min (4°C) using a Beckman Coulter centrifuge (Avanti JXN-26, Mississauga, ON, Canada) and the pellet was resuspended in 8 ml of normal saline to make the final concentrated SE LS101 challenge.

All the birds were challenged with 0.5 ml of SE LS101 by gavaging orally using a reusable stainless feeding needle (curved, 16 G, 4" length, 3 mm ball diameter: Braintree Scientific Inc., Braintree, MA). Cloacal swabs were taken from all the birds using sterile cotton swabs on the day before the oral challenge of SE LS101. Spleen and caecal contents were collected at -2, 1, 4 and 7 d relative to the oral SE challenge. Samples were collected from one bird per cage at each day of the collection after euthanizing by cervical dislocation. Spleen and caecal content from one caecum of each bird were aseptically excised from the carcass and put into two separate pre-weighed normal saline-containing (10 and 5 ml respectively) sterile plastic centrifuge tubes. The tube weights were recorded after collecting samples. The tubes containing caecal content were vortexed and then serially diluted (10-fold) up to 10^{-5}. Each dilution of caecal content (100 µl from each dilution) was plated on Brilliant Green agar and incubated at 37°C overnight. The tubes containing spleen were homogenized using a homogenizer (Fisher Scientific PowerGen 125, Loughborough, UK), and 100 µl of each sample were plated directly on Brilliant Green agar and incubated at 37°C overnight. Pink colour colonies were counted in the plates containing number of colonies between 20 and 200. The homogenized spleen samples (1 ml from each tube) were placed into culture tubes containing 5 ml of Selenite Cystine broth and incubated in a shaker at 37°C overnight for enrichment. The enriched samples were streaked on Brilliant Green agar plates, incubated at 37°C overnight and then recorded for the presence or absence of pink colour colonies in Brilliant Green agar plates. Cloacal swabs were directly plated on Brilliant Green agar, incubated overnight at 37°C and the presence or absence of pink colour colonies was recorded.

The Brilliant Green agar plates that are identified as positive for *Salmonella* before the SE LS101 oral challenge was submitted to Prairie Diagnostic Services, Saskatoon, SK, Canada for the identification of species and serovar of *Salmonella*.

7.3.7 Statistical analysis

Data were analyzed using a two-way analysis of variance (2 × 2 factorial arrangement) of the SAS 9.4 Proc glimmix model to determine the main effects of, and interaction between HB and BGase (SAS 9.4, Carey, N.C. 2008). The experiment was a Randomized complete block design since the treatments were blocked by cage level at both experimental facilities. Bacteriological data (counts of colony-forming units and the number of positive samples) were log-transformed before statistical analysis. Differences were considered significant when $P \leq 0.05$. Tukey-Kramer test was used to detect significant differences between means.

7.4 Results

7.4.1 Dietary fibre

Total dietary fibre, IDF, SDF and total β-glucan in HB were 29.1, 19.3, 9.8 and 8.03%, respectively. The same parameters in wheat were correspondingly 16.1, 13.6, 2.5 and 0.75%. Hulless barley was consisted of 52.1% total starch, 16.6% CP, 2.4% fat and 1.8% ash, whereas wheat contained 65.6% total starch, 15.4% CP, 1.4% fat and 1.8% ash.

7.4.2 Salmonella Enteritidis colonization and translocation

Salmonella (confirmed as *Salmonella* Infantis) was isolated from cloacal swabs, caecal content, and spleens of broiler chickens representing all treatments at the pre-challenge. Hulless barley reduced the number of CFU of *Salmonella* by faecal shedding before challenge, but enzyme addition did not affect *Salmonella* shedding (Table 7.2). Neither HB nor BGase level nor their interaction affected caecal *Salmonella* colonization before or after challenge with SE (Table 7.3). However, HB numerically ($P = 0.13$) reduced the number of *Salmonella* colonizing the caeca at the pre-challenge, which is supportive of *Salmonella* fecal-shedding results. *Salmonella* (CFU) was absent in the spleen when considering the direct culture in all the treatments at each collection point. However, HB reduced the percentage of birds positive for *Salmonella* in the spleen at pre-challenge ($P = 0.06$) and d 1 of post-challenge, whereas increased the values at d 4 of SE post-challenge (Table 7.4).

Table 7. 2. Effects of hulless barley and β-glucanase on the percentage of Salmonella- positive cloacal swabs in broiler chickens prior to challenge with Salmonella Enteritidis

Hulless barley (%)	β-glucanase (%)	*Salmonella*-positive cloacal swabs (%)
0	0	11.8
	0.1	20.0
60	0	3.2
	0.1	0.1
SEM[1]		2.98
Main effects		
Hulless barley (%)		
0		15.9[a]
60		1.6[b]
β-glucanase (%)		
0		7.5
0.1		10.0
Probability		
Hulless barley		0.01
β-glucanase		0.66
Hulless barley × β-glucanase		0.31

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

[1]SEM - pooled standard error of mean (n=40 birds per treatment).

Table 7. 3. Effects of hulless barley and β-glucanase on Salmonella colonization in the caeca of broiler chickens

Hulless barley (%)	β-glucanase (%)	*Salmonella* colonization (log CFU/g of caecal contents)			
		Pre-challenge d -2	Post-challenge d 1	Post-challenge d 4	Post-challenge d 7
0	0	3.2	3.7	1.9	1.6
	0.1	3.1	2.9	1.8	1.3
60	0	2.2	4.8	3.0	0.4
	0.1	1.5	4.0	1.4	1.5
SEM[1]		0.42	0.37	0.39	0.32
Main effects					
Hulless barley (%)					
0		3.2	3.3	1.9	1.4
60		1.9	4.4	2.2	1.0
β-glucanase (%)					
0		2.7	4.2	2.5	1.0
0.1		2.3	3.5	1.6	1.4
Probability					
Hulless barley		0.13	0.16	0.68	0.42
β-glucanase		0.66	0.30	0.27	0.44
Hulless barley × β-glucanase		0.75	0.97	0.31	0.27

[1]SEM - pooled standard error of mean (n=10 birds per treatment).

Table 7. 4. Effects of hulless barley and β-glucanase on the percentage of broiler chickens positive for Salmonella in the spleen (after enrichment)

HB[1] (%)	β-glucanase (%)	Pre-challenge	Post-challenge d 1	Post-challenge d 4	Post-challenge d 7
0	0	68.7	70.0	45.6	65.8
	0.1	41.1	70.0	67.3	38.3
60	0	20.8	40.0	78.9	58.9
	0.1	29.1	20.0	98.1	69.8
SEM[2]		7.84	8.00	7.14	8.11
Main effects					
Hulless barley (%)					
0		54.9	70.0[a]	56.4[b]	52.1
60		25.0	30.0[b]	88.5[a]	64.4
β-glucanase (%)					
0		44.8	55.0	62.2	62.3
0.1		35.1	45.0	82.7	54.1
Probability					
Hulless barley		0.06	0.01	0.01	0.45
β-glucanase		0.51	0.51	0.08	0.61
Hulless barley × β-glucanase		0.23	0.51	0.91	0.24

[a-b]Means within a main effect not sharing a common superscript are significantly different ($P \leq 0.05$).
[1]HB - hulless barley
[2]SEM - pooled standard error of mean (n=10 birds per treatment).

7.4.3 Short chain fatty acids and intestinal pH

There were no significant effects of HB and BGase on caecal SCFA and pH of broiler chickens at d 14 (Table 7.5 and 7.6). However, both the concentration and molar percentage of isobutyric acid tended to increase with the addition of HB to the diets ($P = 0.08$). Further, caecal pH was lower with BGase ($P = 0.07$) compared to without BGase in the diets.

Table 7. 5. Effects of hulless barley and β-glucanase on caecal short chain fatty acids of broiler chickens at 14 days

HB[1] (%)	BGase (%)	SCFA µmol/g of wet caecal content								Molar percentage of total SCFA						
		Total	Acet	Prop	Buty	Isob	Val	Isov	Cap	Acet	Prop	Buty	Isob	Val	Isov	Cap
0	0	249.0	155.2	55.9	25.5	5.0	0.0	5.1	1.5	62.3	22.4	10.4	2.0	0.0	2.0	0.5
	0.1	259.3	159.8	58.3	26.9	3.3	1.7	6.9	2.1	61.6	22.5	10.3	1.3	0.6	2.6	0.8
60	0	252.7	153.6	55.6	25.7	7.4	3.2	6.3	0.5	60.7	22.0	10.1	2.9	1.2	2.5	0.2
	0.1	256.4	157.2	57.3	26.6	5.5	1.7	6.7	1.1	61.3	22.3	10.4	2.2	0.7	2.5	0.4
SEM[2]		5.41	3.40	1.33	0.60	0.67	0.55	0.62	0.34	0.30	0.16	0.06	0.26	0.21	0.23	0.13
Main effects																
HB (%)																
0		254.1	157.5	55.8	26.5	4.1	0.8	6.0	1.8	61.9	22.5	10.4	1.6	0.3	2.3	0.7
60		254.5	155.4	57.8	26.2	6.5	2.4	6.5	0.8	61.0	22.1	10.3	2.5	0.9	2.5	0.3
BGase (%)																
0		250.8	154.4	57.1	25.9	6.2	1.6	5.7	1.0	61.5	22.2	10.3	2.4	0.6	2.3	0.4
0.1		257.9	158.5	56.5	26.8	4.4	1.7	6.8	1.6	61.4	22.4	10.3	1.7	0.6	2.6	0.6
Probability																
HB		0.97	0.76	0.81	0.85	0.08	0.15	0.68	0.14	0.12	0.32	0.36	0.08	0.13	0.64	0.19
BGase		0.53	0.56	0.46	0.46	0.17	0.92	0.41	0.40	0.83	0.59	0.61	0.17	0.89	0.53	0.37
HB × BGase		0.77	0.94	0.89	0.96	0.94	0.15	0.57	0.92	0.28	0.69	0.24	0.99	0.17	0.53	0.88

[1]HB - hulless barley; BGase - β-glucanase; SCFA - short chain fatty acids; Acet - Acetic acid; Prop - Propionic acid; Buty - Butyric acid; Isob - Isobutyric acid; Val - Valeric acid; Isov - Isovaleric acid; Cap - Caproic acid.
[2]SEM - pooled standard error of mean (n=10 birds per treatment).

Table 7. 6. Effects of hulless barley and β-glucanase on caecal pH of broiler chickens at day 14

Hulless barley (%)	β-glucanase (%)	pH
0	0	6.00
	0.1	5.97
60	0	6.11
	0.1	5.77
SEM[1]		0.050
Main effects		
Hulless barley (%)		
0		5.99
60		5.94
β-glucanase (%)		
0		6.05
0.1		5.87
Probability		
Hulless barley		0.61
β-glucanase		0.07
Hulless barley × β-glucanase		0.12

[1]SEM - pooled standard error of mean (n=10 birds per treatment).

7.4.4 Performance parameters

The interaction of HB and BGase was significant for body weight gain from d 0 to 14. Beta-glucanase increased the body weight gain of broiler chickens when given a wheat-based diet, but not with an HB-based diet. Neither HB nor BGase affected feed intake. Both HB and BGase decreased feed to gain ratio, although the interaction was not significant. The total mortality of the experiment was 1.3%.

Table 7. 7. Effects of hulless barley and β-glucanase on production performance of broiler chickens aged d 0-14

Hulless barley (%)	β-glucanase (%)	Body weight gain (kg)	Feed intake (kg)	Feed to gain ratio
0	0	372.7[b]	493.1	1.32
	0.1	403.4[a]	515.4	1.27
60	0	393.1[ab]	508.8	1.29
	0.1	394.1[ab]	498.7	1.26
SEM[2]		3.51	3.69	0.01
Main effects				
HB (%)				
0		388.1	504.3	1.30[a]
60		393.6	503.7	1.27[b]
β-glucanase (%)				
0		382.9	501.0	1.30[a]
0.1		398.8	507.0	1.27[b]
Probability				
HB		0.38	0.94	0.03
β-glucanase		0.01	0.39	0.0009
HB × β-glucanase		0.02	0.20	0.35

[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 (n=10 cages per treatment).

7.5 Discussion

Pre-challenge sample collection of caeca, spleen and cloacal swabs was completed to confirm the birds were free of *Salmonella* before the oral challenge with SE. However, *Salmonella* was isolated from the spleen, caeca and cloacal swabs, and identified as *Salmonella* Infantis in all the treatments. It is a multi-drug resistant bacterium that is often found in chickens and chicken food products during outbreaks (Nogrady et al., 2012; Franco et al., 2015). The broilers were confirmed as *Salmonella* negative at day 1 of the experiment and they were managed according to standard bio-security measures until moving to the Bio-security level 2 facility. Therefore, it is evident that the contamination of *Salmonella* Infantis is associated with a biological cause rather than treatment associated. Interpretation of the results regarding

Salmonella colonization in the caeca and translocation to the spleen in broiler chickens should be made with caution since the pre-challenge *Salmonella* contamination was reported in the current study. The pre-challenge *Salmonella* Infantis might have modified the microbial composition in the GIT and affected further colonization with SE during the challenge. The SE colonization degree might be different than in *Salmonella*-negative birds due to the *Salmonella* Infantis associated bacterial dysbiosis in the broiler chickens, which could affect further colonization and growth of pathogenic bacteria (Chow et al, 2010; Kogut, 2019)

There was significantly less percentage of birds isolated for *Salmonella* Infantis in the cloacal swabs when the birds were fed an HB- compared to wheat-based diets, which is a contrast to the hypothesis of the current study. In general, soluble non-starch polysaccharides, including barley β-glucan, are thought to increase the opportunity for colonization of anaerobic micro-organisms in the chicken small intestine (Kaldhusdal and Hofshagen, 1992; Malthlouthi et al., 2002; Józefiak et al., 2010). It has been associated with high intestinal viscosity that leads to reduced feed passage rate in the absence of β-glucan digesting enzymes in feed when chickens are fed a barley-based diet (White et al., 1983; Salih et al., 1991; Almirall and Esteve-Garcia, 1994). It disrupts the microbiome balance in chicken digestive tract and increases the opportunity to colonize more pathogenic microbiota in GIT including *Salmonella*. Therefore, a reduction of *Salmonella* counts with an HB-based diet is contradictory to the viscosity induced microbial colonization in the digestive tract of chickens. In agreement with the current work, high MW β-glucan in HB decreased *Salmonella* colonization in pigs when they were challenged with *Salmonella* Typhimurium (Pieper et al., 2012). However, digestion of β-glucan in barley is different in pigs compared to poultry since pigs have a comparatively long digestive tract, and well-developed caeca and colon for the digestion of fibre (Bach Knudsen et al., 2012). Further, very high and complete β-glucan digestion at the end of the pig small intestine have been shown even with the absence of feed BGase (Fadel et al., 1988; Bach Knudsen and Hansen, 1991). Moreover, the reduction of *Salmonella* colonization in the broilers fed high β-glucan HB in the previous research was associated with the changes in colonic SCFA molar ratios; increased propionic acid and decreased acetic acid (Pieper et al., 2012). Further, it has been observed ileal acetic acid increases expression of Salmonella Pathogenicity Island 1 (SPI-1) invasion genes,

whereas colonic propionic or butyric acids decreased the expression of SPI-1 genes (Lawhon et al., 2002), which supports the effect of SCFA profile on *Salmonella* shedding and translocation in pigs. However, there were no treatment effects on SCFA levels in the current study to relate this concept to chickens.

Salmonella colonization increased at d 1 post-challenge in the caeca, and then gradually decreased as shown by the means (CFU) of the treatments (pre-challenge - 2.5, d 1 - 3.9, d 4 - 2.0, d 7 - 1.2). However, *Salmonella* colonization in the spleen (% of birds) increased until d 4 post-challenge, and then decreased (pre-challenge - 39.9, d 1 - 50, d 4 - 71.7, d 7 - 56.7). Hulless barley significantly affected the spleen *Salmonella* colonization despite a lack of significant differences in caecal colonization. *Salmonella* colonization in the spleen decreased with HB at pre-challenge ($P = 0.06$) and d 1 post-challenge, which is in accordance to the observation for cloacal swabs at pre-challenge. In contrast, the spleen *Salmonella* colonization increased with HB at d 4 post-challenge. Further, it was numerically increased again at d 7 post-challenge. Hulless barley might have delayed the spleen translocation of *Salmonella* at the pre-challenge and early post-challenge (d 1) of SE possibly due to a beneficial effect of HB β-glucan. It might be associated with the 'stimbiotics' concept of soluble fibre which causes signaling the fibre degrading intestinal microbiota to effectively digest fibre (Bedford, 2018; Ribeiro et al., 2018; Gonzalez-Ortiz et al., 2019) since the broilers were fed HB-based diets for a long period of time in the current research. This adaptation might increase the production of SCFA in the lower GIT, which affects *Salmonella* colonization in the caeca and translocation to the spleen (Van Immerseel et al., 2006). In contrast, *Salmonella* recovery in the spleen was higher in the treatments of HB-based diets compared to wheat-based diets at d 4 post-challenge of SE. However, the difference was lower compared to pre-challenge and d 1 post-challenge. The contrasting results at d 4 might be due to the increased *Salmonella* translocation to the spleen with increasing duration of the SE post-challenge.

Short chain fatty acids are the primary products of carbohydrate fermentation and have multiple effects on host energy metabolism and GIT microbiota (den Besten et al., 2013; Jha et al., 2019). Short chain fatty acids may reduce intestinal pH in chickens and thereby cause the reduction of acid-sensitive pathogenic bacteria in GIT by dissipating protein motive force across

the cell membrane of bacteria (van Der Wielen et al., 2000). Dietary fibre, including barley β-glucan, positively changes the digestive tract microbial population by shifting protein fermentation towards saccharolytic fermentation (Józefiak et al., 2010). In particular, butyrate which produced as a result of carbohydrate fermentation can reduce *Salmonella* invasion in the intestinal epithelium by affecting *Salmonella* invasion genes (Gantosis et al., 2006; Van Immerseel et al., 2003). In addition, butyrate provides energy for the growth of intestinal epithelial cells (Sakata and von Engelhardt, 1983; Kripke et al., 1989) that results in improvement of GI barrier, which prevents entering pathogenic microbes into the systemic circulation through GI wall (Peng et al., 2009; Wu et al., 2018). However, previous research did not observe an increased butyrate production as a result of feeding HB in poultry. Further, total and other main SCFA (acetate and propionate) production results were not consistent in chickens fed HB and feed BGase (Józefiak et al., 2005; 2006).

Previous research in this thesis (Chapter 3, 4, 5 and 6) has shown BGase depolymerizes high MW β-glucan into lower MW polysaccharides in the ileal soluble digesta of broiler chickens. Further, endoxylanase supplementation increased water-soluble fraction of arabinoxylan with the age of broiler chickens, which demonstrates the gut microbial effect on degrading arabinoxylan in wheat (Bautil et al., 2019). It was hypothesized the reduction in MW of non-starch polysaccharides would enhance fermentation and the production of SCFA. Caecal pH was lower with the addition of dietary BGase compared to the treatments without BGase (P = 0.07), which might indicate increased carbohydrate fermentation in the caeca. However, caecal SCFA in the current study were not affected by dietary treatments and this is in agreement with previous research that observed inconsistent effects of dietary BGase on SCFA when the broilers were fed barley-based diets (Józefiak et al., 2005; 2006). Dietary BGase increased the concentrations of total SCFA and propionic acid in Józefiak et al. (2005), while no BGase effect was observed according to Józefiak et al. (2006) for SCFA levels in the caeca. Although SCFA are considered an important factor affecting intestinal pH, there are many other factors that affect GIT pH in chickens including feed ingredient composition (proteins and minerals), protein fermentation metabolites (ammonia, biogenic amines, indoles and phenols), and physiological condition of the birds (Apajalahti, 2005).

Performance variables were within the normal range for the production period of d 0-14 according to Ross 308 Broiler Performance Objectives (Aviagen, 2014). Overall, BGase increased the body weight gain of broiler chickens before the SE challenge. Further, dietary HB and BGase increased the feed efficiency of broiler chickens. The BGase effect on improving the production performance might be associated with the reduction of digesta viscosity (Salih et al., 1991; Fuente et al., 1995; Yu et al., 1998) and resulting increased nutrient digestibility (Edney et al., 1989; Brenes et al., 1993; Ravindran et al., 2007). The reason for HB improved feed efficiency in comparison to wheat is less obvious. Hulless barley contains a higher fibre content (TDF - 29.1%, SDF - 9.8% and β-glucan 8.0%) than the wheat (TDF - 16.1%, SDF - 2.5% and β-glucan - 0.75%), which has previously been found to negatively affect broiler performance (Classen et al., 1985).

In conclusion, HB reduced the pre-challenge fecal shedding of *Salmonella*, and *Salmonella* translocation to the spleen at pre-challenge ($P = 0.06$), and d 1 post-challenge of SE in broiler chickens. However, HB increased the spleen recovery of *Salmonella* at d 4 SE post-challenge of the birds. The mechanism of the HB effect on *Salmonella* colonization and translocation in broiler chickens is not clear in the current research and warrants further investigation.

8.0 GENERAL DISCUSSION

Antibiotics have been used in poultry feed to improve poultry production by increasing growth promotion, feed conversion and preventing enteric diseases in chickens for decades (Engberg et al., 2000). However, the prophylactic use of specific antibiotics in poultry feed has been banned or reduced all over the world due to the regulatory compliance (European Commission, 2005; Chicken Farmers of Canada, 2019) and public awareness regarding the emergence of antibiotic-resistant genes in chickens and humans (Diarra et al., 2007; Roth et al., 2019), which affects food safety and public health (Smith et al., 2002; Marshall and Levy, 2011). However, the susceptibility to enteric and zoonotic diseases in chickens increases with the removal of prophylactic use of antibiotics in the poultry industry. Therefore, it is imperative to investigate alternatives to in-feed antibiotics in the poultry industry since other disease control strategies including bio-security measures and vaccination programs are not fully effective at maintaining poultry health (Diarra and Malouin, 2014). Nutritional alternatives play a major role in terms of alternatives to antibiotics since feed affects the gastro-intestinal (GI) physiology and health of chickens in many ways.

Extensive research has been completed on feed additives including prebiotics, probiotics, symbiotic, essential oils as alternatives to antibiotics in chickens (Ducatelle et al., 2015; Gadde et al., 2017). Nevertheless, most of the feed additives must be used in combination to obtain more effective results (Arsi et al., 2015; Mundt et al., 2015), and are often specific in controlling a certain type of pathogenic microbiota (Fernandez et al., 2002; Eeckhaut et al., 2008) in the poultry gastro-intestinal tract (GIT), which leads to a high cost of production and less practical use in the industry. Further, research findings have also been inconsistent concerning most of the antibiotic-alternative products used in poultry production.

Prebiotics are defined as “a substrate that is selectively utilized by host microorganisms conferring a health benefit” by the International Scientific Association of Probiotics and Prebiotics (Gibson *et al.*, 2017). Research has investigated the effects of prebiotics, especially fermentable carbohydrates including mannan-oligosaccharides, fructo-oligosaccharides,

arabinoxylo-oligosaccharides and xylo-oligosaccharides on performance and GI barrier function of chickens.

Dietary fibre is an important component in poultry feed that has been investigated in research concerning prebiotics in antibiotic-free production. Soluble fibre including arabinoxylan and β-glucan are found in common cereal grains used in poultry feed. They are not digested in the small intestine and reached the lower digestive tract of chickens. However, the addition of specific exogenous non-starch polysaccharidases depolymerizes the soluble fibre into more fermentable substrates and facilitates entry into the caeca, which is the main site of fermentation in poultry. The entry of fermentable substrates into the caeca is restricted to small, less viscous and soluble material, which further supports the importance of fibre-degrading enzymes in the feed (Choct et al., 1996; 1999) to increase the ability of fermentable material to exert prebiotic effects in chickens.

The current research was designed to assess the effects of β-glucan (level of hulless barley; HB) and pure β-glucanase (BGase) levels on β-glucan depolymerization in the small intestine and subsequent production and physiological responses. Feed BGase is used in the poultry industry when the diets are mainly based on barley, since it has been proven that exogenous BGase reduces digesta viscosity and improves growth performance, nutrient digestibility, and colonization of beneficial GI microbiota in chickens. Most of the previous research related to exogenous enzyme use in barley and oat poultry diets used mixed enzyme sources that contain endo-xylanase and BGase activity, although the enzyme effect was often attributed to BGase. Further, there are very few poultry studies that investigated individual and synergistic effects of BGase and xylanase when fed barley-based diets. Dos Santos et al. (2013) found that a reduction of digesta viscosity (cPs) with individual enzymes and a higher degree of response with the use of both enzymes (control - 4.38^{a}; xylanase - 3.28^{bc}; β-glucanase - 2.81^{cd}; combination - 2.56^{d}), while no improvement in performance. Similarly, an *in vitro* study showed a reduction of barley viscosity (ml/g of DM) with the use of individual enzymes and more with the combination (control - 9.91^{a}; xylanase - 3.80^{b}; glucanase - 1.62^{c}; combination - 0.50^{d}) (Mathlouthi et al., 2002b). This is the first time that purified BGase has been used in barley

research to investigate its dose effect on β-glucan depolymerization, chicken production performance and digestive tract characteristics.

8.1 Ileal soluble beta-glucan molecular weight distribution

This is the first time in the literature that barley β-glucan molecular weight (MW) has been studied in the digesta of chickens. However, it has been studied in pigs, and the reduction of soluble β-glucan Mw was from 1050×10^3 Da to 460×10^3 Da (diet to the duodenum) and from 1050×10^3 Da to 250×10^3 Da (diet to the ileum) without addition of BGase into the barley-based diets (Holtekjølen et al., 2014). The comparatively high β-glucan MW in the ileum might be associated with increased utilization of low MW β-glucan in the small intestine of pigs and only the high MW β-glucan is remained for the fermentation in the lower digestive tract. The current study found β-glucan Mw values of 649×10^3 Da and 625×10^3 Da for HB-based diets without and with BGase vs 72×10^3 Da and 48×10^3 Da in the ileum digesta for the same treatments. It suggests the MW of β-glucan is reduced even without addition of exogenous BGase and this might be associated with the process of diet digestion and/or digestive tract bacterial enzymes.

Overall, exogenous BGase depolymerized high MW soluble β-glucan in HB in a dose-dependent manner and this resulted in lower MW β-glucan in the ileum of broiler chickens and laying hens. The supplementation of BGase in the diets reduced the Mp, Mw and MW-10% of soluble β-glucan molecules in the ileal digesta of chickens in all the experiments. In addition, exogenous BGase depolymerized β-glucan in wheat, although wheat contains a low amount of total β-glucan compared to HB (wheat; 0.64-0.75%, HB; 8.03-8.70%). Therefore, the first part of the overall hypothesis in the current study is accepted since exogenous BGase dose resulted in an increased amount of low MW soluble β-glucan, which theoretically is a readily available substrate for fermentation in the lower GIT of chickens. However, the enzyme dose-dependent reduction of ileal soluble β-glucan MW was more consistent and larger for HB compared to wheat, and it might be associated with the structural difference of β-glucan in wheat and HB. Wheat has a high DP3:DP4 compared to barley and it is β-glucan with high or low DP3:DP4 that is considered to render β-glucan less soluble and prone to aggregation, which may potentially make β-glucan less susceptible to exogenous BGase.

The response of β-glucan MW to exogenous BGase is consistent across the trials as shown by BGase reducing the MW parameters and increasing the proportion of low MW β-glucan as shown by curve placement relative to the blue line at x-axis point $1e^4$ in each curve (Figure 8.1). However, BGase addition resulted in a bimodal distribution curve (a separate larger MW peak other than the Mp) only for the broiler chickens challenged for coccidiosis at both d 11 and 33 (Figure 8.2B) and not for the broiler chickens fed diets with BGase and housed in battery cages (Figure 8.1B) or laying hens fed HB-based diets with BGase (Figure 8.3B). The reason for the second large MW peak is unknown but may be associated with the release of insoluble β-glucan that has not yet depolymerized or aggregation of lower MW β-glucan. The former rationale could be explained by changes in digestive tract microbiota that has a different capacity to release insoluble β-glucan from the grain cell wall. The species of *Eimeria* in the coccidiosis challenged birds may affect the colonization of other gut microbiota in the digestive tract compared to un-challenged birds and affect the degradation of non-starch polysaccharides. Moreover, the larger peak might also be related to the variability in gut microbial community due to the differences in age and housing. It is noteworthy that cage-housed birds do not have access to their own excreta or an extensive environmental microbiota as birds housed in litter floor pens. However, it is difficult to interpret the association of the second MW peak to the aggregation of lower MW β-glucan since it is only noted in the experiment of coccidiosis challenged broilers.

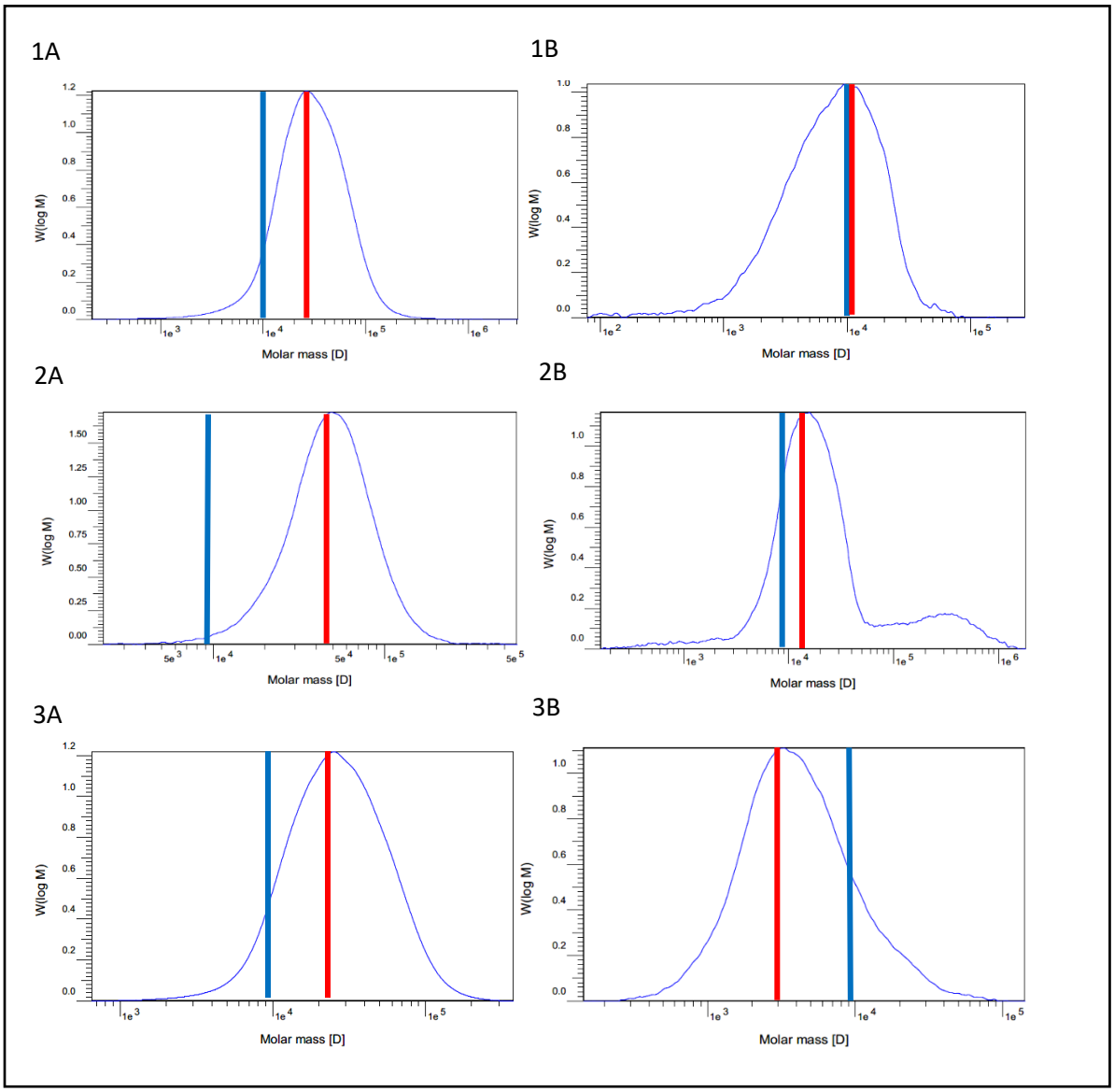

Figure 8. 1. Beta-glucan molecular weight distribution in soluble ileal digesta from chickens fed hulless barley-based diets. Blue lines denote point 1e4 on the x-axis and red lines indicate the Mp of the distribution curve. 1A. 60% hulless barley and 0% β-glucanase; 1B. 60% hulless barley 0.1% β-glucanase (cage-housed broiler chickens), 2A. 60% hulless barley and 0% β-glucanase; 2B. 60% hulless barley 0.1% β-glucanase (coccidiosis challenged broiler chickens), 3A. 73% hulless barley and 0% β-glucanase; 3B. 73% hulless barley 0.1% β-glucanase (laying hens).

8.2 Digestive tract characteristics and production performance

Intestinal pH was measured in the current study as an indication of SCFA production related to carbohydrate fermentation in chickens. In general, BGase increased ileal pH and decreased caecal pH in broilers. It suggests that BGase shifts the fermentable, small MW carbohydrates from the ileum to caeca (Choct et al., 1999) and it might be associated with the reduction of mean retention time of digesta in the ileum due to the increased β-glucan solubility with BGase-induced β-glucan depolymerization in the ileal digesta (Schop et al., 2019). However, SCFA concentrations in the ileum and caeca did not always correspond to pH values, which were consistent across the experiments. The variable and inconsistent SCFA results in the study may reflect the less accuracy of estimating SCFA production based on collecting digesta at a single time. A portion of SCFA get absorbed into the portal circulation or utilized by the digestive tract bacteria and produce other metabolites, thus the analyzed concentration of SCFA in the ileum and caeca might not accurately indicate SCFA production in chickens. Further, caecal evacuation might have occurred in different times of chickens, although the samples were collected in a specific time frame to avoid bias. Therefore, the variability of caecal emptying might affect caecal SCFA concentration in chickens and the very least, this introduces variability into the data set and reduces the possibility of statistically significant differences.

Hulless barley increased the proportional empty weights and lengths of digestive tract sections and digesta content, which agrees with the increased soluble fibre content in HB. According to the current research, total dietary fibre, soluble dietary fibre and total β-glucan of HB ranges were 26.7-29.1, 7.8-9.8 and 8.0-8.7 respectively. Soluble fibre, including β-glucan examined in this experiment, is known to reduce nutrient digestibility due to increased digesta viscosity and other factors, thus the GIT increase is a compensatory mechanism to increase the efficiency of nutrient digestion. The digesta content increase may be associated with decreased feed passage rate due to high viscosity as well as more dry matter as a result of low nutrient digestibility. Beta-glucanase decreased GIT size since BGase increases the nutrient digestibility and there is no requirement to maintain a larger digestive tract. Hulless barley associated larger digestive tract might be related to the gut hormones secretion in the small intestine. The

undigested nutrients and SCFA in the digestive tract activate small intestinal L-cells to produce GLP-I, GLP-2 and PYY (Hiramatsu et al., 2005; Aoki et al., 2017; Miyamoto et al., 2018). The hormones (GLP-I and PYY) are thought to reduce gastric emptying and feed passage rate, while GLP-2 increasing cell growth in the intestines.

An objective of the current thesis was to depolymerize HB β-glucan using exogenous BGase and determine its biological impact. The research in chapter 4 suggests that young birds, at least those recently challenged for coccidiosis, responded negatively to increased levels of lower MW β-glucan. In contrast, in older birds, performance increased, possibly due to an increased capacity to utilize depolymerized β-glucan and affect digestive tract function due to more of a diversified and beneficial GI microbiota that develop with the increasing age of birds.

8.3 Diet medication

Medication reduced the MW of soluble ileal digesta β-glucan in broilers fed diets without exogenous BGase, which suggests an increase in microbial BGase responsible for depolymerizing high MW β-glucan. Antibiotics modify the microbial population in the GIT of chickens (Simon et al., 2016; Xiong et al., 2018) resulting in microbota with an increased capacity to utilize high MW β-glucan and produce lower MW polysaccharides and oligosaccharides. Bacterial depolymerization of β-glucan in the digestive tract of chickens is supported by the isolation and identification of anaerobic bacteria which can degrade mixed-linked β-glucan in broilers (Beckmann et al., 2006). Medication consistently decreased the caecal SCFA of broiler chickens. Reduced carbohydrate fermentation might be associated with antibiotic mediated alteration of GIT microbiota (Torok et al., 2011; Lin et al., 2013) and the reduction in non-starch polysaccharidases producing bacteria. However, the reduction in fermentation seems in contrast to lower MW soluble β-glucan in the ileal digesta as a result of medication induced depolymerization of high MW β-glucan.

Diet medication decreased the empty weights, lengths and digesta content of GIT in general. The use of specific diet medication decreases pathogenic bacterial colonization in the digestive tract of chickens through the modification of microbial diversity and relative abundance, and immune status, which leads to increased nutrient digestibility. In addition,

nutrient digestibility also increases due to the increased bacteria that has ability to utilize non-starch polysaccharides with the use of feed antibiotics, which is supported by BGase-mediated depolymerization of β-glucan in the current research. Therefore, the necessity to maintain a larger digestive tract is minimum with the use of antibiotics in poultry diet.

Medication increased the growth performance of broilers despite the supplementation of BGase in the diets. However, the effect was higher when the diets did not contain BGase. It reveals the capability of feed BGase to decrease the requirement of antibiotics in diets to improve growth performance. Beta-glucanase partially alleviated the effects of diet HB in broilers challenged for coccidiosis, which suggests that BGase is a significant component of the strategy to replace in-feed antibiotics in the poultry industry. The positive effect of diet medication on growth performance is associated with the modification of gut microbiota that results in a diversified and potentially beneficial microbiota. Further, it might beneficially affect GI health and immune status, and subsequently increases broiler production.

Both diet medication and BGase effects on production performance and digestive tract characteristics were larger in litter floor-raised birds than cage-raised broilers. The broilers raised on litter floor pens were challenged with *Eimeria* spp and had a comparatively more exposure to pathogens due to the litter that contact with excreta, whereas the broilers in battery cages were non-challenged and had a minimum exposure to environmental pathogens. Therefore, the coccidiosis challenged birds might encounter more changes regarding performance, physiology and microbial population compared to the broilers that were housed in cages. It appears research on alternatives to antibiotics including prebiotics will be more successful in disease challenged birds rather than healthy birds.

8.4 Future research and implications

Overall, the inconsistency of carbohydrate fermentation results in the current study suggests the need for investigation of other mechanisms regarding prebiotics in chickens including bacterial competitive exclusion and improvement of immune function. Yeast and fungal β-glucan positively influence the immune system of chickens by binding to pattern recognition receptors located on the immune cells, and activating NF-κB transcription factor

which initiates secretion of inflammatory cytokines, and other immune mechanisms in chickens (Ross, 2000; Taylor et al., 2002). Further, cereal β-glucan also affects immune function of rats and mice and also in *in-vitro* experiments (Davis et al., 2004; Hong et al., 2004; Volman et al., 2010), thus more investigation regarding the effect of cereal β-glucan on the immune function in monogastric animals including poultry is necessary. Further, the investigation of the most effective form of β-glucan as a fermentable carbohydrate or having any other properties including an immune stimulant will be beneficial to the poultry industry and for future research.

The use of semi-purified barley β-glucan rather than feeding barley diet with exogenous BGase, might have a beneficial effect in terms of improving GI physiology, morphology, and production performance of chickens. The usage of semi-purified β-glucan requires investigation on the optimum β-glucan MW and the proportion of oligosaccharides that increase the response criteria including performance, fermentation, and the colonization of beneficial microbial population in the digestive tract. If barley-based diets are fed with exogenous BGase to improve GI characteristics, then the most effective level of BGase that optimize carbohydrate fermentation is important since the current study observed many BGase dose-dependent effects on β-glucan depolymerization, growth performance and GI morphology of chickens. Therefore, the evaluation of more exogenous enzyme dosages is suggested in the chickens fed HB-based diets. The solubility of β-glucan, and the interaction of β-glucan with other fibre types (eg-arabinoxylan) and nutrient components are important criteria regarding the digestibility of β-glucan in GIT of broilers. The cell wall of cereals especially barley consists with a complex structure that β-glucan interacts with other non-starch polysaccharides, protein and phenolic compounds (Burton and Fincher, 2014), and it might affect the location and degree of β-glucan depolymerization and solubilization in the GIT of chickens. Therefore, a detailed analysis of fibre profile in the diets and digesta in different locations of GIT helps in understanding the exact site of the digestive tract that β-glucan solubilization is completed. In addition, the examination of digesta viscosity and β-glucan MW distribution of digesta in different sites of the digestive tract is important concerning the location of β-glucan solubilization. Furthermore, quantification of the amount of total and soluble β-glucan in the digesta is useful to examine the exact sites of β-glucan digestibility and solubilization in the digestive tract of chickens. Moreover, the

quantification of β-glucan in the digesta permits assessing the exact quantity for each β-glucan oligosaccharide or polysaccharide of specific MW, which helps in determining the proportion of β-glucan with specific MW that enhance poultry performance and health. Furthermore, the analysis of the effects of HB and BGase, and medication on the distribution of microbial population of GIT especially in the ileum and caeca is suggested since it will be helpful to connect the effect of small MW carbohydrates including β-glucan, and medication on carbohydrate fermentation and other physiological effects in GIT that eventually contributes to the production performance of chickens. Future research on other fermentable components in poultry feed that improve GI barrier function and growth performance in chickens will also contributes to the identification of more alternatives to antibiotics in poultry production.

8.5 Conclusions

In conclusion, exogenous BGase depolymerized high MW β-glucan in HB in a dose-dependent manner in GIT by the time it reaches the ileum of broiler chickens and laying hens. Consequently, the low MW β-glucan increased the production performance of old broiler chickens even though the young birds were unable to utilize them effectively in terms of improving growth performance probably due to less mature digestive tract and, less well established and diversified GI microbial population. The effect of the low MW β-glucan on carbohydrate fermentation in lower GIT was not consistent across the experiments and not completely clear, although it was speculated carbohydrate fermentation shifted from the ileum to caeca of broilers based on the intestinal pH and SCFA concentrations in some experiments. However, there were fewer effects of HB and BGase on GI physiological and morphological findings in broiler chickens and laying hens. Further, medication resulted in low MW β-glucan by depolymerizing high MW β-glucan of HB in broiler chickens. Medication reduced the carbohydrate fermentation in the caeca possibly through shifting GI microbial population, however it increased the growth performance of coccidiosis challenged broiler chickens. Nevertheless, feed BGase decreased the necessity of antibiotics in HB-based diets concerning the production performance of broiler chickens, which is beneficial in an antibiotic-free production and is suggested as a partial replacement for prophylactic antibiotics in chickens.

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