CYTOHISTOLOGICAL, PHYSIOLOGICAL, AND NUTRITIONAL EFFECTS OF A COMMERCIAL β-MANNANASE PRODUCT IN DUCKLINGS 1-21 DAYS OF AGE

A Dissertation

by

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ABSTRACT

Nutritional formula is an economical key factor to raise poultry. β-mannanase breaks the non-starch polysaccharide (NSP) backbone chains in plant-based feed, then NSP is divided into mannose or mannan-oligosaccharide (MOS). Any study about the utilization of MOS or β-mannanase on the ducks was not conducted to our knowledge. This study was performed to evaluate effects of MOS and β-mannanase on the ducks. Effects of MOS supplementation on live performances started to show at d 21. There were no effects by additional YCW-MOS in intestine length, weight, index, and viscosity. However, YCW-MOS showed its effectiveness on gut morphology and cell formation. YCW-MOS only influenced cysteine, histamine, and tryptophan digestibility. β-mannanase showed its effect on live performance throughout the experiment. β-mannanase showed its effectiveness on organ length, viscosity, and gut morphology and cell formation. β-mannanase not only affected amino acid digestibility, but also affected body and bone composition. Titanium (IV) Oxide was used to test the effect of β-mannanase on digesta passage rate. β-mannanase was found to have a great effect on digesta passage rate. Addition of β-mannanase showed faster digesta passage rate because β-mannanase had influenced viscosity and pH of digestive tracts. In conclusion, the β-mannanase influence proved to be more effective than MOS to ducks. This result seems to be due to the fact that MOS is a derivative of β-mannanase. Therefore, the addition of β-mannanase can be an important factor that duck producers must take into account if they want to earn better profit.

DEDICATION

I dedicate this dissertation to the Lord who helped me during this journey.

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NOMENCLATURE

BW	Body weight
cFCR	Cumulative Feed Conversion Ratio
d	Day
FC	Feed Consumption
FCR	Feed Conversion Ratio
pFCR	Phase Feed Conversion Ratio
MOS	Mannan-Oligosaccharides
min	minutes

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CHAPTER I

INTRODUCTION

Humans have raised and hunted waterfowl for centuries. As evidence, some paintings and carvings were discovered in the Egyptian tombs. The records of humans raising ducks can be dated back to the Roman Empire. There is evidence a Roman, Marcus Porcius Cato, suggested that duck feed formulation should consist of wheat, barley, grape marc, and even sometimes lobster or other aquatic animals (Cherry and Morris, 2008). In China, there are several records that ducks were raised about 1500 years before they began to be raised in Europe. The pottery ducks from the New Stone Age (4,000 and 10,000 years ago) were found in southern China (Wucheng, 1988). The Chinese had already successfully begun breeding Pekin ducks around A.D. 1368-1644 (Jung and Zhou, 1980). These records reflect in reality. China produces about 68% of the world Pekin ducks. Currently, most of the duck meat is produced from Asia (90 %), and followed by others including Europe (11 %) or Egypt (1.67 %) (International Poultry Council, 2013). As duck meat consumption has increased worldwide, the production efficiency has become more important than in the past. Nutrition could be a critical economic factor because the diet cost accounts for more than 70 % of poultry raising. Therefore, the determination of adequate nutrition for a duck is necessary to ensure its good health. Zeng et al. (2015) studied how different levels of dietary energy and protein impacted ducks. Duck diets contained similar nutrients as chickens', but energy concentration was different. Simply, the duck starter diet contained less metabolizable energy (ME) (Kcal/kg), more protein (%), and more amino acids (%) than broiler chicken diets. However, the duck grower diets

contained more ME (Kcal/kg), less protein (%), and less amino acids (%) than broiler chicken diets.

American Pekin duck was derived from Chinese mallard duck and is the most popular duck breed in the United States. Most duck farms use pelleted corn-soybean based feeds for ducks. Corn does not have an impact on digestibility or viscosity of digesta, but soybean has a chance to induce poor digestibility by poultry species because soybean contains about 6 % sucrose, 1 % raffinose, and 5% stachyose (Leeson and Summers. 2005). Therefore, the corn-soybean-based diet contains plant polysaccharides that are also well known as non-starch polysaccharides (NSPs). Mannan is the main components of the plant polysaccharides that are hard to digest by monogastric livestock. NSPs are repeating units of mannose using β-1, 4 linkages. The NSPs can lead to several adverse effects on monogastric animals; 1) reducing the glucose absorption (Sambrook et al., 1985), 2) decreasing nitrogen retention (Kratzer et al., 1967), 3) interfering with IGF-1 secretion (Nunes and Malmiof, 1992), 4) decreasing rate of gastric emptying (Rainbird and Low, 1986), 5) increasing intestinal viscosity (Dale, 1997), and 6) increasing waste of energy by stimulating the innate immune system (Zhang and Tizard, 1996). All effects mainly caused by increasing intestinal viscosity lead to decreased digestibility and negative modification of gut morphology. Therefore, the duck feed or other poultry feed needs an enzyme that can break the mannan linkages to make mannan-oligosaccharides (MOS) or mannose. β-Mannanase is one of the enzymes that can be a solution for breaking the linkages of mannan in NSPs. The residues of NSPs by the β-mannanase are mannose and

MOS. MOS and mannose have a similar effect, such as modifying gut morphologies (villi, crypt, and the goblet cells).

MOS can be found in yeast cell wall surface. Most commercial MOS dietary supplement products in the United States are derived from *Saccharomyces cerevisiae* yeast cell wall. The yeast cell wall mainly consists of β-1,3 (30-45 % of wall mass)/1,6-glucans (5-10 % of wall mass), mannan-oligosaccharides (MOS, 30-50 % of wall mass), or nucleotides (Klis et al., 2006). There are *–O* and *–N*-glycosyl protein groups on the yeast cell wall that can be developed as MOS (Kath et al. 1999). Simply, *N*-glycosylated proteins receive an oligosaccharide through an *N*-glycosidic bond, and *O*-mannosylated proteins receive short mannose chains through an α-mannosyl bond (Lesage et al., 2006). Then it becomes α-(1,2)- and α-(1,3)-D-mannose branches or along α-(1,6)-D-mannose chains (Spring et al., 2015; Vinogradov et al., 1998). MOS and mannose are well known as a pathogen inhibitor. MOS and mannose also reduce pathogen activity in the gut, such as gastro colonization. For example, gram-negative pathogenic bacteria membrane can be bound to the MOS protein conjugates instead of binding on the host's intestinal epithelial cell (Ferket et al., 2002). Mannose also binds type-1-fimbriae of *Salmonella* (Spring et al., 2015). The *Salmonella* bound mannose will be expelled with *Salmonella* through the animal vent. Therefore, the pathogens go through the host's intestine without colonization. MOS protein conjugates also can be linked to host immune cells that lead to enhancing the immune system (Wismar et al., 2010). Many researchers also found that β-mannanase, MOS, and mannose have effects on increasing lymphocytes and reducing heterophils in poultry species (Zou et al., 2006; Mehri et al., 2010; Lourenco et al., 2015). To our

knowledge, experiments that utilized mannan-oligosaccharides (MOS) from yeast cell wall only or β-mannanase only on ducks do not exist. Therefore, the effect of the dietary β-mannanase product on broiler duck live performance, and mucosal morphological development will be evaluated based on several studies.

CHAPTER II

REVIEW OF LITERATURE

Introduction

Over the past several decades, knowledge of the poultry diet formulation has been significantly improved. After antibiotic usage was inhibited in animal feed worldwide, many research projects were performed to find alternative feed additives. Enzymes and prebiotics are some of the most well-known feed additive products that can substitute antibiotics. To begin with, non-starch polysaccharides (NSPs) are main anti-nutritional components of poultry feed. NSPs are well known to inhibit nutrient utilization in monogastric animals. Monogastric animals' digest NSPs much less than ruminants. NSPs are known to cause increasing viscosity of the digesta, and to modify micro intestinal environments. Therefore, NSPs reduce digestibility and interrupt nutrient absorption. The enzyme supplement can be the solution. Through many studies, β-mannanase, that breaks mannan backbone, is reported to improve animal live performance (Ferreira et al., 2016), intestinal environment (Karimi et al., 2015), and reduce intestinal viscosity (Lee et al., 2003). When β-mannanase breaks the mannan backbone, mannan-oligosaccharides (MOS) and mannose are created; MOS is one of the popular prebiotic feed additives for poultry. Therefore, MOS is the by-product of the mannan linkages that are the main components of the NSPs. Several researchers found that yeast derivative MOS in commercial products influenced the population of lymphocytes and neutrophils (Lourenco et al., 2015), intestinal morphology (Jahanian et al., 2016), and reduced several pathogens (Santos et

al., 2012), such as *E. coli, salmonella,* or *C. perfringens*. (Spring et al., 2000; Mostafa et al., 2015; Fowler et al., 2015).

Pekin duck diets

Interest in duck diets has increased as with the increasing consumption of duck meat. Commercial Pekin ducks for meat are raised for about 45-56 days. They have a much bigger body than broiler chickens, and also consume a lot more feed than broiler chickens. Optimum levels of ingredients and nutrition composition are important for improving production cost. The optimum broiler chicken diet formulation was found through many studies. However, research about duck dietary energy level is still ongoing. Because absorption abilities of various nutrients in duck are very different than that of chickens. Kong and Adeola (2013) compared amino acid digestibility of broiler chickens and Pekin ducks. The author concluded that broiler chicken diet cannot be the same as duck diets because ducks have higher amino acids losses than broiler chickens. There are also experiments about determination of energy level in duck diet. Fan et al. (2008) used diets with six different energy levels for 14- to 42-day old Pekin ducks. This study showed body weight increased as dietary energy level increased. The author concluded 3008 or 3030 kcal/kg and 18% of crude protein (CP) were most ideal levels for Pekin duck diets. Xie et al. (2010) studied five different energy levels in Pekin ducks. That study showed live performance was improved by increasing dietary energy level. However, high energy diet did not impact breast and leg meats. As dietary energy level increased, so did percentage of fat in the body. The author concluded 3016 kcal/kg is most ideal energy level on day 1 to 21. Zeng et al. (2015) used three different dietary metabolizable energy

(ME) and three different crude protein (CP) concentrations from 15 to 35 days. The author found there was correlation between ME and CP on live performance. Live performance was improved by increasing ME and CP. Through this study, the author concluded 3284 kcal/kg and 19% of CP is most ideal energy level for the grower phase (15-35 days) of Pekin ducks. However, a few European companies suggested to use lower ME (2900-2980 kcal/kg for starter and 3050-3150 kcal/kg for grower) and CP (19.5-20% for starter and 17-19% for grower) than the above publications (Orvia Rearing guide for commercial Pekin duck, Grimaud Freres Rearing guide for roasting Pekin duck). Therefore, the controversy about duck dietary energy level is not expected to stop soon. The duck diet is not only important for improving production efficiency, but also correlated with natural hormones. Farhat and Chavez (1999) found that high protein diet fed Pekin ducks had more Insulin-Like Growth Factor-I. Therefore, modification of the duck diet will be a very important factor to induce improved live performance.

Amino acids for duck diets

The proper amount of amino acids in poultry diet is critical for poultry growth. Ingredient and nutrients for duck diet to maximize the growth of ducks have not been developed and researched well by the closed duck industry. Therefore, there is not much data on proper amino acid levels in duck diets, so efforts to find the optimum amount of amino acids in duck diets have been ongoing until recently. Some authors mentioned the NRC data are too old and there are some big differences between duck species because of different growth rates (Bones et al., 2002; Swatland, 1980). Also, amino acid levels for broiler chicken diet formulation is not even possible to use for duck diets because ducks

have higher amino acids losses than broiler chickens (Kong and Adeola. 2013; Jamroz et al., 2001). Therefore, the optimum amino acid levels for modern duck diets should be reinvestigated and reevaluated.

Effects of β-mannanase in livestock

β-mannanase is a popular commercial enzyme feed additive product, which gained popularity after antibiotics were banned from use on livestock. Mannan is major component of hemicellulose in the plant cell wall. β-mannanase, the mannan degrading enzyme, breaks down mannan backbone to mannan-oligosccharides (MOS) or other fermentable sugar (mannose etc.) through endohydrolases and exohydrolases processing (Moreira and Filho., 2008). Most livestock feed contain some mannan. The efficacy of β-mannanase on the growth of poultry species has been found through many experiments. β-mannanase is not only helpful to monogastric livestock, but also is helpful to ruminants, such as cows and goats.

Lee et al. (2014) studied the effect of β-mannanase on Korean native goat. In this study, three different levels (0, 0.1, and 0.3%) of β-mannanase were used. There was no significant difference in dry matter intake, the highest dry matter, and organic matter digestibility among treatments. However, the β-mannanase treated group had significantly greater weight gain, feed conversion ratio, and nitrogen retention. Another study supports the same idea. Lee et al. (2010) studied the effect of β-mannanase on calves. The author used 0.1% of commercial β-mannanase product with 3 and 8% of palm kernel meal. The β-mannanase treated group trended to have increase feed intake. There were no significant

differences in *E. coli* population in the gastrointestinal tract. Overall, this study showed that 8% of palm kernel meal with 0.1% of β-mannanase is ideal concentration for calves.

Palm kernel meal is emerging as a replacement for corn-soybean meal. However, the palm kernel meal contains 30-35% of mannan. Therefore, including palm kernel meal can be a critical issue for poultry diets. To solve this problem, Lee et al. (2013) used laying hens to study the effect of β-mannanase on palm kernel meal. Two levels (0 and 5%) of palm kernel meal with or without of the β-mannanase were used in this study. Both palm kernel meal and the β-mannanase treated group had significantly improved egg production. Albumen height was increased in the β-mannanase treated group. Therefore, the β-mannanase will be helpful for countries that imports more than 90% of its grain in order to produce feed for livestock. The positive effect of β-mannanase on guar meal, another corn-soybean meal substitute that consist of 65% of mannose and 35% of galactose (Kok et al., 1999), was identified through many studies. Lee et al. (2003) studied the effect of β-mannanase on ileal digesta viscosity of broiler chickens. The experiment used two different types of guar meal and three different levels of the β-mannanase. The author found that not only did the β-mannanase treated group have significantly reduced intestinal viscosity, but increased body weight and reduced feed conversion ratio. Mohayayee et al. (2011) studied the β-mannanase effect on different levels (low 2, 4, and 6%; intermediate 4, 6, and 8%; high 6, 9, and 12%) of guar meal (germ fraction). At result, the intermediate level of guar meal with the β-mannanase treated group had significantly greater body weight gain, feed intake, feed conversion ratio, carcass and giblet indices, and plasma lipids than other treatment groups. However, there was no effect of the β-mannanase on

the high level of guar meal. Therefore, the β-mannanase worked on the intermediate level guar meal inclusion. Daskiran et al. (2004) researched the effect of β-mannanase through two different experiments; one evaluating different level of guar gum (0, 0.5, 1, and 2%), and the other concerning different levels of the β-mannanase (0, 0.5, 1, and 1.5%). In the first experiment, the authors found that the β-mannanase treated group had significantly improved feed efficiency, but dietary metabolizable energy (ME) and net energy were numerically increased. In the second experiment, the β-mannanase treated group had significantly improved feed conversion ratio at d 14.

There are many kinds of enzyme products for poultry, but few have studies shown that the β-mannanase is the most effective enzyme on poultry. Ayoola et al. (2015) used turkeys to compare effects of the β-mannanase only and multi-enzyme (blend of xylanase, amylase, and protease). Both treated groups showed reducing apparent endogenous loss of nutrients caused by the significant reduction of ileal adherent mucin thickness layer. The β-mannanase treated group had significantly increased jejunum width, surface area, and villi height and crypt depth ratio than the control group. The β-mannanase also had effects on live performance and production of laying hen. Wu et al. (2005) studied effect of the β-mannanase on second cycled leghorns. In this experiment, high energy diet, low energy diet, and the β-mannanase with low energy diet were used. According to the result, feed conversion ratio of low energy diet with the β-mannanase had similar result as the high energy diet. There was a significant increase in egg production and egg mass from the low energy diet with the β-mannanase treated group from week 5 to 8 of the study.

However, there were no significant differences on feed intake, egg specific gravity, egg weight, mortality, and body weight.

Many experiments have been done to find the proper concentration of β-mannanase in poultry diets. Jackson et al. (2004) used four different concentrations (0, 50, 80, and 110 MU, MU = 106 enzyme activity units) of commercial β-mannanase product (Hemicell, ChemGen Corp.) on corn-soybean meal diet for broiler chicken. The 80 MU/ton treatment had higher weight gain and feed conversion ratio than other concentration. Mussini et al. (2011) used four different levels (0%, 0.025%, 0.05%, and 0.1%) of the β-mannanase. As a result, the digestibility of Lysine, Methionine, Threonine, Tryptophan, Arginine, Leucine, Isoleucine, Cysteine, and Valine, and ileal apparent metabolizable energy were significantly improved. From another experiment (Mussini et al., 2011), the β-mannanase treated group had no significant difference in live performance, but β-mannanase significantly reduced dry matter excreta output per bird. This result also showed the trend that nitrogen level in feaces was decreased as the level of the β-mannanase increased in the diet. Therefore, the β-mannanase had a positive effect on nitrogen utilization. The β-mannanase also increased calcium and phosphorus level. On the other hand, Latham et al. (2016) could not find any effect of β-mannanase on ileal digestible energy and viscosity. The author studied effects of the β-mannanase in reduced energy diet on broiler chickens. In that experiment, a high energy diet, a low energy diet, and the β-mannanase with low energy diet were used. According to result, the β-mannanase treatment of the reduced energy diet could achieve live bird performance similar to the positive control group.

Generally, the β-mannanase is well-known to impact poultry live performance. Kong et al. (2011) used the commercial β-mannanase dietary supplement that significantly improved the apparent total tract utilization of dry matter, nitrogen, and apparent metabolizable energy in the broilers. Early stage (d 0 to 22) of birds had significantly higher body weight gain by the β-mannanase, but grower stage (d 23 to 44). Imran et al. (2014) studied different dietary energy levels with the β-mannanase on broilers. The β-mannanase treated group had significantly improved body weight, gut morphology, feed conversion ratio, and immunity, but there was no significant difference in feed intake and mortality. Klein et al. (2015) studied effect of the β-mannanase and NSPase. That experiment found that β-mannanase only treatment, NSPase only treatment, or even β-mannanase/NSPase treated groups improved live performance of broiler chickens. Barros et al. (2015) studied effect of a growth promoter, β-mannanase, and MOS. However, there was no significant difference between each group. Rather, β-mannanase + MOS group had lowest value of body weight gain at d 42. β-mannanase also impacted poultry gut morphologies. Karimi et al. (2015) compared effect of the β-mannanase and β-glucanase on intestinal morphology in male broilers with various levels of metabolizable energy. At result, the β-mannanase and the β-glucanase treated group had significantly greater duodenal villus length, width, crypts depth, jejunal villus length, crypts depth, illeal villus length, width, and crypt depth. The β-mannanase also impacted poultry immune system. Jackson et al. (2003) compared the effect of β-mannanase supplementation and antibiotics on broiler chickens with *Eimeria spp.* and *C. perfringens* challenges. Throughout the experiment, the β-mannanase treated group had lower lesion score than the control group,

but not more than those treated with antibiotics. Therefore, the β-mannanase could be replacement of antibiotics. Several experiments showed that the β-mannanase also impacted the chicken immune system. Zou et al. (2006) used four different levels (0, 0.025, 0.05, and 0.075%) of the β-mannanase commercial product. This study showed that there was no significant difference in feed intake during the 0 to 3 week and 0 to 6 week periods, or in immunoglobulin A (IgA) and immunoglobulin G (IgG) populations in serum.

However, the β-mannanase treated group had higher weight gain in 4 to 6 and 0 to 6 weeks. The groups treated with 0.025% and 0.05% of β-mannanase had significantly greater feed conversion ratio than the control group. Immunoglobulin M (IgM) concentration and T lymphocyte proliferation also improved in the 0.05% β-mannanase treated group. The β-mannanase affected the populations of lymphocytes and heterophils too. Mehri et al. (2010) used broiler chickens with four different levels of the β-mannanase (0, 500, 700, 900 g/ton). According to the result, all β-mannanase treated groups had significantly increased villus height, crypt depth, and decreased goblet cell counts in small intestine. The β-mannanase treated group also had significantly increased lymphocyte and decreased heterophil population. However, the β-mannanase did not affect the blood serum proteins, and eosinophil and monocyte populations. Therefore, β-mannanase has effects on the chicken immune system. The β-mannanase also affected the size of immune organs. Ferreira et al. (2016) used four different diets (β-mannanase treated group; normal nutritional requirements of broilers; reductions of 100 kcal metabolizable energy; 3% of the total amino acids; and 100 kcal metabolizable energy and 3% total amino acids) during the study. The β-mannanase treated group had significantly greater body weight gain,

apparent metabolizable energy (AMEn), true ileal digestibility coefficients for all amino acids, reduced nitrogen, immune organ indices (spleen and bursa), and concentration of immunoglobulin A, G, and M in blood serum.

Effects of mannan-oligosaccharides (MOS) in livestock

Mannan-oligosaccharides (MOS) and mannose are by-products that result from breaking the mannan linkages of NSP by β-mannanase. MOS is a commercial prebiotic dietary supplement that has been used for the past decade in poultry nutrition (Spring et al., 2015). Most commercial MOS dietary supplement products are derived from yeast *Saccharomyces cerevisiae* cell walls. The yeast cell wall mainly consists of β-1,3 (30-45 % of wall mass)/1,6-glucans (5-10 % of wall mass), mannan-oligosaccharides (MOS, 30-50 % of wall mass), and nucleotides (Klis et al., 2006). Therefore, MOS in most commercial dietary products are not pure (Fowler et al., 2015).

Antibiotics, especially bacitracin methylene disalicylate, have been used as an animal growth promoter. As with β-mannanase, several MOS studies that compared the effects of antibiotics and MOS in broiler chickens showed no differences between antibiotics and MOS in broiler chicken growth performance. Waldroup et al. (2003) used 0.75 g/kg and 1 g/kg of Bio-Mos (Alltech Inc., Nicholascille, KY) with 55 mg/kg of bacitracin methylene disalicylate and 16.5 mg/kg of virginiamycin. The three different results (antibiotic only, Bio-Mos only, and combination of antibiotics and Bio-Mos) showed that there were no significant differences between MOS and antibiotic treated groups. Hooge et al. (2003) also compared MOS products with antibiotics (bacitracin methylene disalicylate). Both groups showed improvement of body weight, feed

conversion ratio, and net income per bird compared to the control group. Therefore, this research found that the effects of MOS were similar to antibiotics. Flemming et al. (2004) compared the mannan-oligosccharides, *Saccharomyces cerevisiae* cell wall, and a growth promoter (Olaquindox) on broiler chickens. Live performance of the birds fed MOS was significantly higher than the control and the *Saccharomyces cerevisiae* cell wall treated group, but not compared to the growth promoter-treated group. MOS impacted live performance of broiler chickens more than another prebiotic or antibiotic. Yang et al. (2007) found that 2 g/kg of MOS affected body weight gain of the broiler chicken numerically and MOS did not effect the gut morphology at d 14, but was impacted at d 35. Therefore, MOS had impact on only the later stage of broiler chickens. Benites et al. (2008) used two different commercial MOS products with several different concentrations of treatments (Control, 1 kg/ton (starter), 0 kg/ton (grower), and 0.5 kg/ton (finisher) of Bio-Mos, and 0.5 kg/ton (starter), 0 kg/ton (grower), and 0.5 kg/ton (finisher) of SAF-mannan) on broiler chickens. The effect between Bio-Mos and SAF-mannan showed that the Bio-Mos had significantly greater body weight at d 42 than the control group and SAF-mannan treated group. The authors found that SAF-Mannan showed only effects on feed consumption between d 0 and 21. Fowler et al. (2015) found the MOS-treated group had higher growth rate and better FCR under *C. perfringens* challenge. MOS did not effect egg production and quality, but MOS had effects on hatchability and sperm quality. Shashidhara and Devegowda (2003) researched effects of MOS on broiler breeder production and immunity. As a result, MOS did not influence egg production and the proportion of live sperm, but MOS showed significantly higher hatchability with lower

dead-in-shell birds and higher antibody population against infectious bursal disease virus (IBDV). Also, Spring et al. (2000) used *Salmonella* as a challenge and found that MOS did not affect significantly the concentration of cecal coliforms, but results only showed numerical improvement. Iqbal et al. (2015) researched effects of MOS on egg quality and geometry of Japanese quail breeder. There were no significant effects on the yolk index, shell thickness, albumin index, albumin and yolk pH, Haugh unit score, and shape index. MOS was found to effect the host gut morphology by different challenges, such as *Salmonella*. Baurhoo et al. (2007) found that birds fed MOS had significantly higher villi height and number of goblet cells per villus than the control group. The MOS-treated group also had greater numbers of beneficial bacteria (*Lactovacilli*, *Bifidobacteria*) in the ceca and lower population of *E. coli* in the litter than the control group. However, in a different study, yeast cell wall, mannonprotein, or β-1, 3/1, 6-glucans did not significantly impact growth rate of broiler chicken at d 42 significantly (Morales-Lopex et al., 2009). However, the MOS treated group had higher jejunum villus height than the control group. Santos et al. (2012) found that MOS-treated group had lower *Salmonella* population and improved intestinal environment and recovery after infection. Mostafa et al. (2015) used a commercial MOS product (Bio-Mos), and found that birds fed. 0.5 g/kg had higher body weight gain, feed intake, and lower *E. coli* population. Birds fed 1 g/kg had higher jejunal and ileal villus length, lower cecal *Salmonella*. Jahanian et al. (2015) used two different levels of MOS (1 and 2g/kg). As a result, the 2 g/kg treated group showed increased carcass yield, decreased bacterial population by *Aflatoxin* challenge, increased crypth depth, goblet cell counts, lymphoid follicular diameter. MOS also was found to effect the

host immune system. Lourenco et al. (2015) used *Salmonella enteritidis* as a challenge to three different treatment groups; 1) control, 2) broiler chickens were fed 1 kg/ton of MOS on d 1 to 21 and 0.5 kg/ton of MOS on d 22 to 56, and 3) broiler chickens were fed 2 kg/ton of MOS on d 1 to 21 and 1 kg/ton of MOS on d 22 to 56. The author found that the MOS-supplement treated group had more T lymphocyte population than the control group. Arsi et al. (2015) compared fructo-oligosaccharide (0.125%, 0.25%, or 0.5%) and MOS (0.04%, 0.08%, or 0.16%) on *Campylobacter* challenge. However, there were no reductions of *Campylobacter* in both fructo-oligosaccharide and MOS treated groups, but 0.04% of MOS treated group only. In another study, MOS supplementation produced better results to compare with enzymatically-treated palm kernel expeller (PKE) dietary additive. Navidshad et al. (2015) found that the MOS treated group had better live performance than the PKE treated group. However, another study showed MOS did not impact live performance. Al-Sultan et al. (2016) compared effects between probiotic, prebiotic, and symbiotic and showed that prebiotic feed additive had the least effect. Even, another study found that MOS did not impact the digestibility in chicken (Yang et al. 2008). Therefore, MOS can be ineffective without challenges.

Goblet cells

Environments of gastrointestinal microbiota are important to maintain homeostasis of normal host intestinal conditions (Bart and Gaskins, 2016). A basic function of goblet cells is secretion of mucin in intestinal epithelium. Goblet cells secrete mucin in two different ways, either by synthesizing new mucin granules or by releasing stored mucin (Deplancke and Gaskins, 2016). Mucin can be categorized into four different mucin

oligosaccharides; *N*-acetylglucosamine, *N*-acetylgalactosamine, fucose, and galactose. These mucin oligosaccharides contain peptide backbones that consist of glycosylated and nonglycosylated domain with polymer *O*-linked glycosylated regions (Forstner et al. 1995). Lysine plays a role in protein *O*-linked glycosylation (Wu, 2013). The mucin backbone also contains certain amino acids. Threonine, serine, and cysteine have function to establish the mucin backbone (Horn et al., 2009). Especially, threonine has the function of synthesizing the mucin protein and protein phosphorylation and *O*-linked glycosylation in the intestine (Wu, 2013). Horn et al. (2009) performed a threonine deficiency experiment with Pekin duck to find a correlation between mucin secretion and threonine. The author found that mucin secretion was increased by increasing threonine concentration in the duck diet. Goblet cell density and the expression of mucin gene (MUC2) mRNA abundance were also increased as threonine increased. However, the author could not find a correlation between threonine deficiency and mucin secretion in broiler chickens. The author found that sialic acid, one of the by-products from mucin oligosaccharide (Forstner et al. 1995), excretion was increased in broiler chickens.

Mucin can be categorized in two different types, neutral and acidic mucins. Neutral mucin can be found in the large intestine. Several studies showed acidic mucin can be found in the early life stages of humans (Filipe et al., 1989), mice (Hill and Cowley. 1990), and swine (Turck et al., 1993), so acidic mucin is very important for innate immunity because early life stages of the host do not have fully developed cell-mediate immunity (Deplancke and Gaskins, 2016). Also, chicken embryos and hatchlings contain populations of the maternal or endogenic IgA positive plasma cells that exists in poultry

gut, lung, and cloacal bursa. The maternal IgA in embryos is considered to be absorbed from the yolk. Hatchlings have low maternal IgA populations but increase by maturation (Bar-Shira et al., 2013).

When mucin makes contact with water, mucin changes to a gel-like form that is called mucus. Simply, mucus consists about 95% of water and proteins. When pathogen starts colonization of the host gut microflora, dehydration is induced on the host epithelial cell wall (Deplancke and Gaskins, 2016). Dehydration of epithelial cell wall induces modified host intestinal morphology and secretion of mucin by goblet cells that causes nutrient absorption disorder, innate and cell-mediate immune system disorder, and difficulty in protecting from enteric infections (Sun et al., 2013; Bar-shira et al., 2014). When a pathogen occurs on epithelial cells to cause pro-inflammation, interleukin 1 (IL-1) stimulates goblet cell lines to release mucin (MUC genes or HT29-Cl.16E cells) (Deplancke and Gaskins, 2016; Jarry et al., 1996). Tumor necrosis factor α (TNF-α) and IL-6 also stimulate goblet cell lines to secrete mucin genes (*MUC2, MUC5AC, MUC5B,* and *MUC6*). Khan et al. (1995) found CD4+ T lymphocytes appeared in gut parasitic infection that caused inhibition of mucin secretion by goblet cells. Lake et al. (1980) found that immunoglobulin E (IgE) mediated mast cell stimulated goblet cell mucin secretion by discharge of histamine in rat duodenum. Therefore, concentration of histamine in diets has an effect on stimulation of mucin secretion by gastrointestinal tract goblet cells (Wu, 2013). Sun et al. (2013) performed an experiment to conduct correlation between immune challenge and secretory immunoglobulin A (sIgA) by the goblet cells in chicken. Through the study, the author collected duodenum, jejunum, and ileum of chickens to collect

populations of intestinal intraepithelial lymphocytes (IEL), goblet cells, and sIgA. The results showed increased IEL, population of the goblet cell and sIgA in the epithelial lining. A deep relationship and connection between cytokines and mucin secretion by goblet cells has been confirmed through many studies. Mucus helps to protect epithelium from pathogens, lubricate passage of nutrient objects, hydrate the epithelium, and exchange gases and nutrients between the luminal contents and epithelial lining by using their gel-like layer (Bansil and Turner. 2006). However, regulatory reactions or production by the goblet cells are still not defined fully (Bart and Gaskins, 2016). However, the goblet cells in gut microflora have effects on innate and cell-mediate immunity (Gaskins et al., 2016).

CHAPTER III

DIETARY ENZYME SUPPLEMENTATION IN DUCK NUTRITION: A REVIEW

Introduction

Poultry diet formulation has significantly improved over the past few decades as nutrient utilization research has focused on innovative alternative feed additives to improve productive performance. The use of enzymes as feed supplements to improve live performance has been researched extensively in chickens. The broiler and layer chicken industries have used enzymes as dietary supplements for decades. Unlike the chicken industry, there is uncertainty about enzyme usage in duck diets. However, there have been some reports regarding enzymes in duck diets (Table 3.1). The effects of phytase on ducks were studied from the 1990's to the 2010's, while the effects of xylanase on ducks were studied in the early 2000's, and the effects of multiple enzyme treatments on ducks have been studied from the 1990's to the present. However, there are still many questions regarding enzyme usage in duck diets that require answers. For example, the optimal levels of individual enzymes have not been properly established for the formulation of duck diets. Determination of optimal levels of enzymes is important because the level of an enzyme will affect its efficacy and the overall performance of the bird. Although the effects of phytase, xylanase, and multi-enzyme treatments have been extensively researched in ducks, numerous untested enzymes remain. For example, β-mannanase is known to break the mannan backbone, which improves intestinal health in poultry. However, no experiments on β-mannanase have been performed in ducks.

Table 3.1. Effects of enzymes on ducks with dietary ingredients listed

Enzyme	Feedstuffs (plant ingredients)	Impact	References (year)
Phytase	Sorghum and soybean meal	Increased P retention and ash in tibia	Farrell et al. (1993)
Phytase	Molasses, sorghum, wheat, and rice bran/fish meal	Improved AME; increased feed intake, tibia ash and P retention	Martin et al. (1998)
Phytase	Molasses, sorghum, wheat, and rice bran	Improved mineral retention and affected to tibia bone	Farrell and Martin (1998b)
Phytase	Corn, soybean meal, and sunflower meal.	Improved the calcium and plant phosphorus utilization	Rodehutscord et al. (2006)
Phytase	Corn and soybean meal	Phytase effects depend on various levels of NPP	Ei-Badry et al. (2008)
Phytase	Corn, soybean meal, and rice bran.	Phytase shows different effect by NPP levels	Yang et al. (2009)
Phytase	Corn and soybean meal	Improved live performance, bone ash, and mineral retention and digestibility	Adeola (2010)
Xylanase	Wheat, rye, triticale, and soybean meal	Increased feed intake; reduced digesta viscosity	Timmler and Rodehutscord (2001)
Xylanase	Wheat and soybean meal	Xylanase effects depend on various levels of NPP (diet formulation)	Adeola and Bedford (2004)
Protease	Corn and rice bran	Improved egg production, egg weight, and feed conversion ratio	Biyatmoko and Rostini (2016)
Multi-enzyme	Molasses, sorghum, wheat, and rice bran	No enzyme effects on various levels of rice bran diet	Farrell and Martin (1998a)
Multi-enzyme	Corn, wheat middling, and soybean meal	Improved live performance, nitrogen, and amino acid retention	Hong et al. (2002)
Protease/Multi-enzyme	Corn, soybean meal, wheat middling	Improved energy and nutrient utilization/improved only AMEn and TMEn	Adeola et al. (2007)
Multi-enzyme	Corn, soybean meal, wheat by-products/middling	Improved AA and energy utilization	Adeola et al. (2008)
Multi-enzyme	Corn, wheat, and soybean meal	Improved endogenous digestive enzymes	Rui et al. (2012)
Multi-enzyme	Corn, paddy, rice bran, and soybean meal	Improved performance and nutrition digestibility	Kang et al. (2013)
Multi-enzyme	Corn, rice and wheat bran, and soybean meal	Improved growth rate, utilization of nutrients, and bone mineralization	Zeng et al. (2015)
Multi-enzyme	Corn and soybean meal	Decreased triglycerides and LDL cholesterol, increased blood HDL level	Frasiska et al. (2016)

In this review, the conducted studies will be summarized, and the effects of enzymes on ducks and what further studies can be conducted will be discussed.

Basic benefits of enzymes in poultry diets

Exogenous enzymes in poultry diets are known to improve nutrient digestibility (Mussini et al., 2011), egg production (Lee et al., 2013), immune response (Jackson et al., 2004), and gut morphology (Ayoola et al., 2015). Most of the energy sources in poultry diets are derived from plants such as corn and soybean. These and other common ingredients contain several anti-nutritional factors. Animals produce endogenous digestive enzymes, but enzymes that are produced by the host are not fully efficient for digesting all nutrients (Barletta, 2010). For example, poultry species do not secrete endogenous enzymes to hydrolyze non-starch polysaccharides (NSPs), which are a main component of cereal grains. The ability of monogastric animals to digest water soluble NSPs is much poorer than in ruminants (Iji, 1999). These water soluble NSPs form a gel-like material that reduces feed passage rate in the intestine (Ward, 1995). Longer digestion rate causes microbial fermentation in the intestinal area, thus decreasing oxygen and increasing anaerobic bacteria in the intestinal area (Choct, 1997). These bacteria utilize energy and amino acids at the expense of the host (Hedde and Lindsey, 1986; Saunders and Sillery. 1982). This process not only induces intestinal morphology modification but also produces acetic acids (volatile fatty acids) (Hubener et al., 2002). Acids lower intestinal pH and reduce absorption of nutrients such as minerals and fat (Wood and Serfaty-Lacrosniere, 1992). Consequently, cholesterol levels in the blood are increased by the incremental binding of bile salts (Potter, 1995). In addition, NSPs are known to stimulate the host

innate immune system because the host innate immune system recognizes NSPs as a pathogen-associated molecular pattern (PAMP). The innate immune system of vertebrates and plants respond to pathogen invasion through signaling receptors such as toll-like or pattern-recognition receptors. This mechanism in animals is triggered because plants also have microbe-associated molecules similar to transmembrane and intracellular receptors of animals (Ausubel, 2005). The innate immune system is known as 'the first line of defense' of the host body and is the most important immune mechanism, acting before a humoral response initiates an immune response. Stimulation of the innate immune system by NSPs will unnecessarily consume energy from the host. As a result, NSPs causes various negative effects to the host. Enzyme supplements can abate some of these negative effects. Most of the commercial enzymes in the poultry industry are carbohydrases, proteases, and phytase. Carbohydrases break down polysaccharide backbones producing simple sugars. Xylanase, amylase, and β-glucanase are commercial carbohydrase enzymes that are commonly utilized in poultry diets. For example, xylanase is utilized in poultry diets to help break down xylans in wheat. The protease enzymes break down proteins in ingredients such as corn and soybean meal. A typical anti-nutritional factor of proteins in these plants is trypsin inhibitor, which interrupts the trypsin that is secreted by the pancreas. Trypsin inhibitors are partially degraded by heat, but, as they are not completely inactivated, protease can provide additional degradation. Phytase improves mineral absorption availability from plant feed, especially phosphorus. This can reduce the required level of phosphorus sources in diet formulations and aid in reducing phosphorus pollution.

Xylanase

The digestive tracts of monogastric animals self-secrete enzymes to digest feed, but these self-secreted enzymes are not effective for digesting NSPs. Xylan, a component of hemicellulose in plant cell walls, consists of a 1,4-β-linked D-xylopyranose unit as the main chain, and multiple units of xylose that are attached with other substituent groups attached to the main chain (Paloheimo et al., 2010; Nagar et al., 2012). There are several types of xylan chains. Arabinoxylan is the major xylan group in wheat (Coppedge et al., 2012; Knudsen, 2014). Arabinoxylans increase intestinal viscosity, inhibit nutrient digestion, and modify intestinal morphology. Xylanase is a carbohydrase enzyme that degrades xylan and is known to improve live performance and gut morphology in poultry species. Xylanase hydrolyses the xylose backbone releasing xylooligosaccharides (Meng et al., 2005; Paloheimo et al., 2010) and offsets the adverse effects of xylan in poultry diets. Timmler and Rodehutscord (2001) performed the following four studies to evaluate the efficiency of xylanase with five different levels of wheat/rye (%) and triticale (%) in Pekin ducks: Exp1 (with pork lard): wheat 60 (starter), wheat 56/rye 6.6 (grower); Exp 2 (with soybean oil): wheat 51.5/rye 10 (starter), wheat 46.5/rye 20 (grower); Exp 3 (with pork lard): wheat 51.5/rye 10 (starter), wheat 46.5/rye 20 (grower); Exp 4: wheat 53.7/triticale 15 (starter), wheat 38/triticale 35 (grower), and wheat 32.4/rye 25 (starter) with tallow, wheat 19.8/rye 45 (grower) with tallow. In experiments 1, 2 and 3, the live performance of the xylanase-treated groups was not significantly different from the control group. In experiment 3, the xylanase-treated groups had significantly lower jejunal and ileal viscosity compared to the control group. In experiment 4, the author compared

wheat/triticale and wheat/rye diets in ducks, and the wheat/triticale-treated group showed significantly better live performance and viscosity compared to the wheat/rye-treated group. In experiment 4, the ileal viscosity was decreased by xylanase in both wheat/triticale and wheat/rye diets. In conclusion, xylanase did not have a significant impact on duck live performance, but did seem to have an impact on intestinal viscosity. Based on the results, xylanase appears to be most effective when there is no fat such as soybean oil, pork lard, or beef tallow. This appears to be closely related to the results of Xie et al. (2010), who found that the increase of dietary energy from the enzyme did not result in increased breast or leg meat weight, but rather in additional abdominal fat. Increased fat in duck diets does not only increase intestinal viscosity but also negatively impacts duck meat yield. Adeola and Bedford (2004) also reported similar xylanase effects on ducks. They studied the effect of xylanase on six different diets (low- and high-viscosity wheat diets with 0, 1.5, and 3.0 g/kg of xylanase). Xylanase did not impact apparent nitrogen retention, TME, or TMEn, but apparent dry matter retention was increased with increasing concentrations of xylanase. Xylanase also had a positive impact on weight gain and feed conversion ratio at 0-42 and 14-42 days. Xylanase had a significant impact on duodenal and ileal viscosity, with the greatest impact apparent at 1.5 g/kg xylanase in low- and high-viscosity diets. Xylanase also had a significant impact on ileal digestibility (dry matter, fat, starch, and nitrogen) and energy in ducks. Overall, xylanase only shows an effect when it is added into specific feeds (those with lower levels of dietary energy). Unfortunately, there are few experiments that have examined the impact of xylanase on duck live performance. However, several studies have provided

clear evidence that xylanase has an impact on the intestinal environment. In conclusion, xylanase feed supplements can help to prevent the negative effects of NSPs in duck diets.

Protease

The primary reasons for using protease are to improve protein digestion, energy efficiency, and animal productivity. As mentioned above, soybean meal (SBM) is widely used to provide protein in poultry diets. However, SBM contains anti-nutritional factors including lectins, which cannot be digested by monogastric animals (Gitzelmann and Auricchio, 1965; Lalles, 1993; Ghazi et al., 2003). The adverse effects of these anti-nutritional factors can be dramatically reduced by heat during processing, but heating increases processing costs and has the potential to destroy other nutrients in SBM (Sissons et al., 1982; Coon et al., 1990; Ghazi et al., 2003). Exogenous protease is derived from *Bacillus* species, such as *B. subtilisin* and *B. bacillolysin* (Aehle, 2007). Proteases hydrolyse peptide amides into peptides or amino acid residues that are easily absorbed by the host. Several experiments have examined protease impacts on duck diets. Adeola et al. (2006) studied the effects of protease in White Pekin ducks. Three different levels (0, 7,500, or 15,000 U/kg) of protease were added to soybean- and wheat-based diets. Protease-treated groups had significantly improved energy utilization, dry matter, and nitrogen compared to the control group. From measurements of true N retention, protease not only had an impact on the total amount of dry matter output, but apparent and true nitrogen retention was also increased. From estimates of energy retention, AME and TME were found to increase significantly through addition of protease. Kalmendal and Tauson (2012) used 200 mg/kg of protease in broiler chicken diets. Protease-treated groups had

significantly better digestibility of starch, apparent digestibility of fat, and AMEn than the control group. Biyatmoko and Rostini (2016) reported that protease enzyme supplementation in diets affected the productivity of Alabio laying ducks. Five levels (0, 0.1, 0.15, 0.3, and 0.5 %) of protease were used with diets based on rice bran, yellow corn, fish meal, coconut oil, fish oil, corn oil, limestone, and topmix. The author recommended 0.15 % of protease for laying ducks because egg production (hen-day production), egg weight, and feed conversion ratio were all significantly improved at this rate of inclusion. A significant difference was observed in hen-day production among the enzyme-treated groups. The 0.3 % and 0.1 % inclusion rates showed the highest and lowest production percentages, respectively. Egg weight was not significantly impacted by the treatments. There was a significant difference in feed conversion between the protease-treated groups and the control group, but no significant differences were observed among the protease-treated groups. However, protease appears to be ineffective in the presence of *Aflatoxin*. Stanley et al. (2000) used 0.1 % protease in laying hens and observed that protease had no impact on egg production, egg size, and egg shell quality with *Aflatoxin* challenge. These results suggest that protease may have an impact on not only the utilization of energy and nutrients but also on egg production in duck species, provided that other complicating factors such as aflatoxin are not present.

Phytase

Plants occupy the largest portion of feed ingredients in poultry diets. Enormous amounts of phosphorus exist in plant feed materials in the form of phytate, which is difficult to utilize by monogastric animals (Ravindran et al., 1994). The reason why

monogastric animals do not have the ability to hydrolyse phytate is as follows: 1) monogastric animals do not secrete the enzyme that hydrolyses phytate itself (Ravindran et al., 1994), and 2) phytate is composed of strong chemical complexes with metals using multivalent cations that are hard to utilize in the digestive tracts of monogastric animals (Ravindran et al., 1994). In this case, phytase may be a solution as a feed additive in poultry diets. Phytase is one of the first developed enzymes and has had an enormous impact on the enzyme industry. The market size of the enzyme industry was estimated by Paloheimo et al., (2010) to be 550-600 million dollars, of which phytase represents half. Phytase is commonly obtained from *Aspergillus niger*, *Peniophora lycii*, *Schizosaccharomyces pombe* and *Escherichia coli* (Greiner and Konietzny, 2010). The enzymes 3- and 6-phytase are commonly used as animal feed additives to break phosphate resides at the D-3 position of phytate and initiate dephosphorylation at the L-6 (D-4) position of phytate (Greiner and Konietzny, 2010), respectively. After phytase hydrolyses phytate, phosphate, minerals, and myo-inositol will be released, which improves the availability of phosphorus and minerals. However, proper intestinal pH must be established to optimize phytase efficacy (Greiner et al., 1998).

Many experiments have examined the effects of phytase in ducks. Farrell et al. (1993) studied the effect of phytase (1,000 U/kg) in five different duckling diets. Diets 1 to 5 contained 450 g/kg of sorghum and 300, 400, 500, 400, and 300 g/kg of soybean meal, respectively. Diets 1-3 contained 1 g/kg of CaHPO4 (inorganic phosphorus), diets 4 and 5 contained 4 and 7 g/kg of CaHPO4, respectively. Each diet was formulated with or without 850 U/kg of phytase. The author observed that addition of phytase

supplementation significantly improved feed intake and growth rate but not FCR for ducks fed diets 1, 2, and 3. Phytase-treated groups also had significantly increased P retention and tibia ash weight and percentage in diets 1, 2, and 3 and in diet 4, respectively. All phytase-treated groups showed significantly improved phosphorus retention compared to the non-phytase treated groups except for diet 5. Hence, this study showed that the level of phytase used was not sufficient when the diet contained a high amount of inorganic phosphorus.

Farrell and Martin (1998b) conducted two different studies utilizing phytase in duck diets. Experiment 1 was a factorial arrangement of three concentrations of rice bran (0, 200, or 400 g/kg) that induced poor nutrient absorption by young birds, two concentrations of inorganic phosphorus (1 or 3 g) and 0 or 1,000 U/kg of phytase from 2 to 19 d. In diets with no rice bran and 1 g of inorganic phosphorus, the phytase-treated group had significantly better weight gain and less feed intake compared to non-phytase group. These diets did not differ significantly in feed conversion ratio from other groups. Regardless of concentration of inorganic phosphorus, if phytase was present, weight gain and food intake improved significantly (except for 200 g of rice bran and 3 g of inorganic phosphorus without phytase). Phytase-treated groups had increased tibia ash when the diets included rice bran. Increased phosphorus retention was indicated in the phytase diets, but there was no significant difference in phosphorus concentration of tibia ash among the groups. Phytase significantly improved mineral absorption only in diets without rice bran that included 1 g of inorganic phosphorus. Experiment 2 was a factorial arrangement of three concentrations of rice bran (0, 300, or 600 g) and 0 or 1,000 U/kg of phytase fed

from 19 to 40 days. All diets contained 1 g of added inorganic phosphorus. In this experiment, phytase inclusion in the diet significantly improved weight gain, feed conversion ratio, dry matter digestibility, and nitrogen retention. Phytase also significantly improved total tibia ash (g), but there was no difference in mineral percentages in tibia ash. The impact of phytase inclusion in the diet depends on the amount of substrate (phytate) and other ingredient characteristics. Martin (1998) studied phytase inclusion in duck diets with vegetable or animal (fish meal) proteins. In that experiment, 1,000 U/kg of phytase was used initially and was then increased to 1,500 U/kg at day 15. The phytase had no significant effect on live performance of the ducks. The authors noted that phytase positively influenced lysine and threonine digestibility in vegetable protein diets, again indicating that phytase efficacy depends on the ingredients utilized in the diet.

Rodehutscord et al. (2006) examined phytase levels of 0, 1,000 and 10,000 U/kg in duck diets that also contained calcium phosphate at 10 g/kg (week 1 - 3) and 2 g/kg (week 4 - 5). Increasing levels of phytase resulted in significantly greater body weight gain (1-21 d) and a significant difference in body weight at 14 and 35 d. However, there was no significant difference in feed conversion ratio between the control and phytase-treated groups. There are two hypotheses for why the previous experiments (Farrell et al., 1993; Farrell and Martin, 1998; Martin et al., 1998) had different results. The first possibility is that the quality of phytase has changed over the past decade by improved biotechnologies. The second possibility is the difference between mono- and di-calcium phosphates. Since mono-calcium phosphate has more available phosphorus than di-calcium phosphate, the absorption of phosphorus by phytase in the intestine may be better

(Eya and Lovell, 1997). Phytase is well known for affecting phosphorus and calcium absorption through chicken-based studies (Sebastian et al., 1996; Tamim et al., 2004). Phytase also has the same effect on ducks, as shown by the following experiments. Rodehutscord et al. (2006) performed balance studies to evaluate the effect of phytase on the phosphorus and calcium utilization in White Pekin ducks. In the balance studies, two different diets were used as follows: diet (1) 4.4 g/kg of total phosphorus and 2.8 g/kg of phytate P with 0, 250, 500, 750, 1,000, and 1,500 U/kg of phytase, and diet (2) 4.2 g/kg of total phosphorus and 2.6 g/kg of phytate P with 0, 250, 500, 750, 1,000, 1,500 and 2,000 U/kg of phytase. As the amount of phytase increased, phosphorus and calcium excretion decreased significantly, and accretion and utilization were increased significantly in both balance studies. These results indicate that the low levels of phytase-treated groups showed the most effectiveness. The author found that slight differences in intrinsic phytase activity are related to P utilization. The effect of phytase on Hsp70 gene expression, thermal reaction, plasma osmotic pressure, hematological parameters, and some plasma parameters in Muscovy ducks during the summer season were determined by Ei-badry et al. (2008). Three different levels of non-phytate phosphorus (NPP) were used in diets during weeks 1 to 3 (0.25, 0.34 and 0.45 %) and weeks 3 to 11 (0.21, 0. 30 and 0.40 %), with two distinct levels of phytase (0 and 750 U/kg). Phytase induced a significant increase in Hsp70, but there was no significant difference in thermal reaction. The NPP-treated group with 0.40 % phytase had the highest levels of aspartate aminotransferase, alanine aminotransferase, uric acid, and creatinine, but presence or absence of phytase did not have a significant impact on liver or kidney function. Plasma osmotic pressure was

significantly decreased with increasing NPP level and phytase supplementation. In the hematology assay, phytase did not have an impact on white and red blood cells or the percentage of packed cell volume. However, the phytase-treated group had a significantly increased hemoglobin concentration compared to the other groups. As a result, phytase only appears to be affected by temperature. Yang et al. (2009) studied the effect of a recombinant phytase on performance and mineral utilization with non-phytate phosphorus (NPP) in *Jinding* laying ducks. In that study, five different levels (0.18, 0.25, 0.32, 0.38, and 0.45%) of NPP were used with 500 U/kg of phytase (except for the 0.45 % NPP diet which did not contain phytase). The results showed that phytase did not impact live performance in laying ducks. Phytase also did not have an impact on apparent calcium and manganese retention of laying ducks. However, the results also indicated that decreases in NPP content in the diet significantly increased phosphorus retention. Only the 0.18% NPP-treated group had lower Cu and Zn retention than the other groups. The 0.38% NPP-treated group had significantly greater Zn retention than the 0.25 and 0.45 % NPP-treated groups. In the tibia ash and mineral content results, the mineral contents increased NPP except for manganese. Only the 0.38% NPP phytase-treated group showed an effect on zinc. These results were similar to mineral concentration in the plasma results, except for calcium and manganese. The effects of phytase on bone mineralization and live performance of ducks were also verified by Adeola (2010). The author used eight different diets with and without phytase from *Escherichia coli* with a corn-soybean meal based diet in male White Pekin ducks (a low-P negative control, a P-adequate positive control, a negative control with 0.5, 1.0, and 1.5 g of inorganic phosphorus, and 500, 1000, and 1500

U/kg of phytase). The positive control and phytase-treated groups had significantly greater body weight, body weight gain, feed intake, feed conversion ratio, tibia ash, and ileal P digestibility. The effect of phytase was increased along with increasing phytase concentration. However, the effect of phytase increase in the diet was less than the effect of an increase in inorganic phosphorus in the diet. Phytase in ducks is now known to have more effects than in the 1990s; although phytase does not impact the live performance of ducks, it does affect a variety of other areas, and its effect on ducks has been demonstrated over time.

Multi-enzyme treatments

In many cases, multiple enzymes are used to compensate for disadvantages of individual enzymes that are used as animal feed additives. For example, in the case of protease, high fat-containing feeds cannot exert a significant effect. To overcome this inefficiency, protease can be mixed with other enzymes to form a multi-enzyme treatment. Proteases are commonly used in combination with other enzymes to overcome adverse effects that are caused by anti-nutritional factors that are present in plant-derived poultry feeds. Several studies of multi-enzyme treatments in ducks have been conducted. A study in the late 1990s did not find any impact on live performance. Farrell and Martin (1998a) performed a study to evaluate the effect of a cocktail enzyme formed by 1,800 to 2,000 U/g of xylanase, 2,300 to 2,800 U/g of α-amylase, 950 to 960 U/g of β-glucanase, and 1,200 to 1,250 U/g of protease at 0, 200, 300, 400, and 600 g/kg of rice bran in the diet on live performance and viscosity of ducks (species unknown). The study verified that the cocktail of enzymes did not show any impacts on live performance. However, the ileal

viscosity of the duck was decreased by the cocktail enzymes as rice bran increased. In the 2000s, studies were conducted on how multi-enzyme treatments affect nutrient digestibility in ducks and how they react in several different diet compositions for ducks. Hong et al. (2002) determined the effect of three different levels (0, 0.375, and 0.5 g/kg) of multi-enzyme treatment consisting of 4,000 U/g of amylase, 12,000 U/g of protease, and 1,600 U/g of xylanase on starter (days 0 to 14) and grower (days 14 to 42) phase White Pekin ducks. The enzyme-treated group showed better live performance (BW, BWG, FI, and feed efficiency) than the control group. The author concluded that 0.5 g/kg of multi-enzyme treatment showed greater ileal and apparent nitrogen retention, and significantly improved ileal amino acid digestibility and apparent amino acid retention. Adeola et al. (2008) also studied how multi-enzyme treatments (7,500 U/g of protease and 44 U/g of cellulase) affect nutrient and energy utilization in starter and grower diets for White Pekin ducks. In this study, starter and grower ducks were tested with and without enzymes. Differences in nutrient absorption were observed between starter and grower diets, and multi-enzyme treatments also had effects on amino acids and energy utilization in White Pekin ducks. The author concluded that there is a dependent relationship between diet composition and enzymes.

Multi-enzyme treatments have also been tested in Cherry Valley ducks. Rui et al. (2012) found that some endogenous digestive enzymes that were stimulated by a multi-enzyme treatment (10,000 U/g of xylanase, 18,000 U/g of mannanase and 3,000 U/g of glucanase) at the starter (days 1 to 21) phase in Cherry Valley ducks. Specifically, the multi-enzyme treatment had an impact on protease, amylopsin, and pancrelipase levels

during the starter period, but effects of multi-enzyme treatments decreased significantly in the following age group; only the trypsinase level was significantly higher than the control group during the grower phase (days 28 to 42). Multi-enzyme treatment (4,400 IU/kg of endo-1,4-β-xylanase, 4,300 IU/kg of endo-1,3 (4)-β-glucanase, and 2,400 IU/kg of cellulase) also had an impact on the live performance and nutrition digestibility of Cherry Valley ducks, as reported by Kang et al. (2013). The author used the multi-enzyme treatment with a basal diet of corn-soybean and with paddy rice added into the diet. Paddy rice is another corn-soybean substitute that is high in fiber. In that study, the multi-enzyme complex was added to corn-paddy-soybean diets at 1.0 g/kg, resulting in significantly better apparent digestibility of nutrients in ducks. Recent studies have also shown that multi-enzyme treatments are sensitive to diet formulation. Zeng et al. (2015) compared the effects of multi-enzyme treatments (1,100 visco-units of endo-β-1,4-xylanase, 100 units of endo-1,3(4)-β-glucanase, and 500 phytase FTU/kg) on different levels of minerals: a diet formulated following NRC requirements, down-spec 1 (down-spec AME 70 kcal/kg, DAA 2 %, avP 1 g/kg and Ca 1 g/kg) and down-spec 2 (down-spec AME 100 kcal/kg, DAA 2.5 %, avP 1.5 g/kg and Ca 1.2 g/kg). The multi-enzyme treatment with down-spec 1- and 2-treated groups showed similar effects as the NRC requirement-treated group in body weight, feed intake, and weight gain of male Cherry Valley ducks. This study also verified that the multi-enzyme treatment down-spec 1-treated group showed similar effects as the NRC-requirement treated group in the apparent availability of energy (%), dry matter (%), ash (%), calcium (%), and phosphorous (%). However, there were no differences between groups treated with multiple enzymes and the control group on

calcium, phosphorus, and alkaline phosphatase levels in the serum of ducks. Therefore, enzymes seem to be affected by diet formula. The increased cholesterol level in blood is one of the adverse effects incurred by NSPs. However, the multi-enzyme treatment could be a promising solution for this issue. Frasiska et al. (2016) showed that a multi-enzyme treatment could ameliorate this problem. The authors used a multi-enzyme treatment (Allzyme SSF, Alltech Ltd, Nicholasville, KY) with *Gracilaria Sp.* on lipid profiles of Tegal ducks. The multi-enzyme treated group had significantly lower triglyceride and low-density lipoprotein cholesterol levels, but that the multi-enzyme treatments increased blood high-density lipoprotein levels. Therefore, enzymes had a positive impact on cholesterol values in duck blood. Overall, the data showed that the effects of multiple enzyme treatments had similar effects as other individual enzymes. These data also showed that multi-enzyme treatments are influenced by dietary formulas. Therefore, it is necessary to determine what feed formulas can induce maximum effects of multi-enzyme treatments.

Conclusion

A variety of experiments have been performed on ducks to understand the use of supplemental dietary enzymes and their effects. This review identified that these accumulated studies provide evidence that enzymes are valuable tools that bring many benefits to ducks. However, questions remain. Previous enzyme studies on ducks showed that enzymes are sensitive to diet formula because enzymes only show their effects when they are added into specific concentrations and diets. There have been few studies to establish proper concentrations of enzymes in duck diets. Therefore, it will be more

efficient to use multiple enzymes after finding the appropriate concentrations of each enzyme to maximize the enzyme effect in ducks. However, only a few enzymes have been tested for their effects in ducks, such as xylanase and phytase. Finding the right concentrations of supplemental enzymes for ducks is an important future experiment because there are still questions as to which diets could induce synergy with enzymes in duck feed to induce the maximize effects of enzymes. Therefore, more experimental data regarding enzymes on ducks should be collected to achieve ideal diets for ducks. There have also been no experiments that show the impact of enzymes on the duck immune system. NSPs are recognized as an enemy by the host innate immune system in the intestinal lumen. Further studies should evaluate how enzymes affect the innate or humoral immune system of ducks. Studies on the immune system with enzymes will further enhance the potential of the duck industry. Finally, further genetic studies should be performed. The effects of supplemental dietary enzymes on duck genes are still unknown. So far, only one study of enzymes on the duck HSP70 gene has been conducted. Although many studies have been done, many unanswered questions remain regarding the effects of enzymes on ducks. Further studies of the effects of enzymes on ducks are necessary to achieve further development of the duck industry. It is hoped that this review will contribute to the improvement of the duck industry.

CHAPTER IV

EFFECTS OF A COMMERCIAL MANNAN-OLIGOSACCHARIDE PRODUCT ON LIVE PERFORMANCE, INTESTINAL HISTOMORPHOLOGY, AND AMINO ACID DIGESTIBILITY IN WHITE PEKIN DUCKS

Introduction

Over the past few decades, changing attitudes that favor the limited use of antibiotics in animal feeds have prompted significant research in the improvement of poultry diet formulations and poultry nutrient utilization. Prebiotic feed additives have become one of the most popular substitute alternatives for antibiotic additives. Mannan-oligosaccharides (MOS) are one of the popular commercial prebiotic dietary supplements and have been used for decades in poultry nutrition (Spring et al., 2015). Commercial MOS dietary supplement products are derived from the *Saccharomyces cerevisiae* yeast cell wall, which mainly consists of β-1,3 (30-45% of wall mass)/1,6-glucans (5-10% of wall mass), MOS (30-50% of wall mass), or nucleotides (Klis et al., 2006). Therefore, most of the commercial MOS products for animals are not 100% pure MOS (Fowler et al., 2015). Antibiotics were often used as animal growth promoters, however, MOS can also be used for this purpose. Several studies compared the effects of antibiotics and MOS in broiler chickens, and the studies showed no different effects between antibiotics and MOS in growth performance (Hooge et al., 2003; Waldroup et al., 2003). MOS products showed improvements in chicken egg hatchability (Shashidhara and Devegowda, 2003), intestine morphologically (Baurhoo et al., 2007), histologically (Jahanian et al., 2015), and immune system function (Lourenco et al., 2015). MOS products also decrease bacteria population

of *Salmonella* (Mostafa et al., 2015) and *Campylobacter* (Arsi et al., 2015) in the small intestine. However, there has not been a study about the utilization of MOS from *Saccharomyces cerevisiae* yeast cell wall on ducks. Therefore, this study addresses the effectiveness of different levels of MOS dietary supplement on growth performance, intestinal digesta viscosity, morphology, histology, and amino acid digestibility of White Pekin ducks.

Materials and methods

Birds, housing, and diets

For a series of two identical studies (experiment A and experiment B), White Pekin duck eggs were obtained from a commercial source (Maple Leaf Farms, Leesburg, IN). Eggs were incubated to hatch, and ducklings were screened, only healthy ones were selected at the Texas A&M University Poultry Research, Teaching and Extension Center (TAMUPRC). A total of 225 birds were allocated into 0.97 × 0.67 × 0.24 m^3 size battery cage pens, which allowed 0.03 m^3/bird at initial placement. Mixed-sex day-old ducklings were randomly housed with five birds per battery unit. Each treatment was replicated nine times for a total of 45 ducks per treatment. In the experiments, a commercial yeast cell wall product (Safmannan-A, Saf Agri/Lesaffre Feed Additives, Milwaukee, WI) that contained MOS (YCW-MOS) was used. The birds were fed a corn-soybean meal basal diet formulation that was adapted from Zeng et al. (2015) (see Table 4.1).

The experiments consisted of five treatments: 0 g/ton (CON), 250 g/ton (T250), 500 g/ton (T500), 1 kg/ton (T1000), and 2 kg/ton (T2000) of YCW-MOS. The starter (d 0-13) and grower (d 14-21) diets were pelleted and manufactured at the TAMUPRC feed

mill. Each battery cage consisted of two feeders and one water tray and ad libitum supply of feed and water. The lighting was provided 24 hours during first four days and 23 hours for each day until d 21. The starting room temperature of 30 °C was set 48 hours before bird placement. The room temperature was then decreased to 27 °C at d 7 and to 23 °C at d 14. The birds' circumstances and environment of the housing were monitored daily. There was no replacing of the birds during the experiment. These studies were conducted in accordance with an approved animal use protocol from the Institutional Animal Care and Use Committee (AUP: IACUC 2016-0139) of Texas A&M University.

Table 4.1. Experimental diets and nutrient composition

	Starter 1-13 d	Grower 14-21 d
Ingredients, %		
Corn, yellow grain	43.24	55.06
Soybean meal, dehulled solvent	39.58	27.20
Wheat midds	6.00	5.99
DL Methionine	0.36	0.27
L-lysine	0.01	0.08
Fat, blended A/V	5.89	7.88
Limestone	2.66	1.18
Bio-Phos 16/21 P	1.25	1.32
Salt	0.42	0.42
Trace minerals[1]	0.05	0.05
Vitamins[2]	0.25	0.25
Nutrient Composition		
Crude Protein, %	23.99	19.01
ME, kcal/kg	3040	3300
Crude Fat, %	8.08	10.38
Lysine, %	1.33	1.05
Methionine, %	0.70	0.55
Cysteine, %	0.38	0.31
Tryptophan, %	0.30	0.23
Threonine, %	0.90	0.71
Arginine, %	1.61	1.22
Valine, %	1.09	0.87
Calcium, %	1.33	0.75
Phosphorus, %	0.68	0.65
Sodium, %	0.19	0.19
Chloride, %	0.30	0.31

[1] Trace mineral premix added at this rate yields 149.6 mg manganese, 55.0 mg zinc, 26.4 mg iron, 4.4 mg copper, 1.05 mg iodine, 0.25 mg selenium, a minimum of 6.27 mg calcium, and a maximum of 8.69 mg calcium per kg of diet. The carrier is calcium carbonate, and the premix contains less than 1% mineral oil.

[2] Vitamin premix is added at this rate yields 11,023 IU vitamin A, 3,858 IU vitamin D_3, 46 IU vitamin E, 0.0165 mg B_{12}, 5.845 mg riboflavin, 45.93 mg niacin, 20.21 mg d-pantothenic acid, 477.67 mg choline, 1.47 mg menadione, 1.75 mg folic acid, 7.17 mg peroxidase, 2.94 mg thiamine, 0.55 mg biotin per kg diet. The carrier is ground rice hulls.

Growth performance

The body weight data were recorded at d 1, 7, 14, and 21. The feed consumption and feed conversion ratio data were collected on d 7, 14, and 21. Productivity index (PI) was calculated by following the formula:

$$\text{PI} = (100 - \text{Mortality}) \times (\frac{\text{BW}}{1000})/\text{Bird Age}/\text{FCR} \times 100$$

Manure data were collected on d 5, 8, 12, 15, 19, and 21 using collected manure from plates in the bottom of each battery cage.

Collecting samples

Jejunum and ileum were harvested from four birds per pen. Jejunum samples were harvested from the first liver portal vein to the Meckel's diverticulum and ileum samples were harvested from the Meckel's diverticulum to the cecal junction to measure total organ length. To evaluate organ weights and indices, the jejunum and ileum weights were recorded. One bird was euthanized via CO2 for harvesting the distal section of the jejunum and ileum samples to evaluate histomorphology. From one bird, whole digesta from the jejunum and ileum were collected to evaluate intestinal viscosity. From two birds, the whole ileal digesta were collected to evaluate ileal amino acid digestibility.

Viscosity

The samples were evaluated as described by Lee et al. (2003) with minor modifications: 1) samples were centrifuged 4,500 × g for 20 minutes rather than 3,500 × g for 10 minutes, 2) viscometer (Brookfield Cone and Plate Viscometer 4 with a CPE-40, Ametek Brookfield, Middleboro, MA) was spindled at 37.8°C rather than at 40 °C, and read and measured after 20 seconds rather than 30 seconds at 5 rpm.

Histology

The jejunum and ileum samples were washed with phosphate buffered saline three times. Then, samples were stored in 70% alcohol (71001-652, VWR International, Radnor, PA) for 24 hours and were transferred into 10% buffered formalin (16004-114, VWR International, Radnor, PA) until fixed. The fixed samples were duplicated and placed into 2 × 2 cassettes (97000-390, VWR, Radnor, PA). All samples were stained with Alcian Blue pH 2.5 (mucin) at the Texas A&M University Histopathology/Immunopathology Laboratory. A NacoZoomer 2.0-HT Digital slide scanner (C9600, Hamamatsu Photonics K. K, Shizuoka Pref., Japan) was used to evaluate the stained sections at the Gastrointestinal Laboratory, Department of Small Animal Clinical Sciences at Texas A&M University. Scanned files were analyzed with NDP.view2 Viewing Software (Hamamatsu Photonics K.K, Shizuoka Pref., Japan) to measure villi height, width, and crypt depth of the jejunum and ileum from two birds. Ten of jejunum and ileum villi were randomly selected to evaluate villi height, width, and crypt depth. The villus width was measured below half of its height.

Digestibility

Titanium (IV) oxide (248576, Sigma-Aldrich, St. Louis, MO) (5 g/kg) was used in grower diets as an indigestible marker to analyze amino acid digestibility. A lyophilizer (FD4, Thermovac, Island Park, NY) was used to dry-freeze ileal digesta samples. The samples were sent and analyzed by the Agricultural Experiment Station Chemical Laboratories at University of Missouri-Columbia. The following formula was used to

calculate the amino acids digestibility coefficients (AAD) as described by Iyayi and Adeola (2014):

$$\text{AAD} = \left\{1 - \left(\frac{\text{Titanium (IV)Oxide (diet)}}{\text{Titanium (IV)Oxide (ieal)}} \times \frac{\text{Amino Acid (diet)}}{\text{Amino Acid (ieal)}}\right)\right\}$$

Statistical analysis

All pooled data of both experiment A and B were analyzed via a 5 (treatments) × 2 (experiments) factorial analysis of variance with using the Standard Least Squares procedure and completely randomized block design in the JMP Pro® 12.0.1 for Windows (SAS Institute Inc., Cary, NC). The data means were separated using the Least Square Means Differences Student's T-test and deemed significantly different at $P \leq 0.05$.

Results and discussion

Growth performances

To investigate the effects of YCW-MOS on ducklings, mortality, average body weight (g), weight gain (g), the cumulative and phase feed conversion ratio, the amount of manure (g), and the productivity index were evaluated. Two mortalities from T500 were observed from Experiment A; and four mortalities were observed from Experiment B: one from the CON, one from the T250, and two from T1000. Therefore, YCW-MOS did not impact mortality of the ducklings.

Table 4.2 presents results of the body weight per bird (BW) and weight gain (WG). Addition of YCW-MOS into diets did not influence BW and WG significantly. T500 and T1000 consumed significantly less (P = 0.0269) feed compared to CON at d 21. An interaction between the treatments and experiments was observed to be significant (P =

0.0385) in d 7 WG. There were no significant differences in WG among the groups at d 7 in either experiment A or B (data not shown).

Table 4.3 presents results of the feed conversion ratio (FCR) and productivity index (PI). T1000 had significantly lower pFCR (P = 0.0456) and cFCR (P = 0.0198) compared to CON and T500 at d 21. A significant interaction (P = 0.0006) was also observed in pFCR at d 1 to 7. T2000 had significantly lower pFCR compared to CON, T500, and T1000 in experiment B (Figure 4.2). No significant differences in FCR were observed at d 7 and 14. However, T1000 had significantly lower FCR (P = 0.0198) compared to CON and T500 at d 21. There were no significant differences in PI at d 7 and 14, but T1000 and T2000 had significantly higher (P = 0.0179) PI compared to CON and T500 at d 21. A significant interaction between treatments and experiments was observed in PI at d 7 (P = 0.0126). T1000 had significantly greater PI values compared to T250 and T2000 in experiment B (Figure 4.3).

The growth performance data showed a slightly different trend compared to several other experiments with broiler chickens.

Table 4.2. Effect of YCW-MOS on weights per bird (g), weight gain per bird (g), and feed consumption per period (g) from d 1-21 in Pekin ducks

Treatment[1]	Body weight (g)				Weight gain (g)			Feed Consumption (g)		
	d1	d7	d14	d21	d7	d14	d21	d7	d14	d21
CON	56.04	273.45	789.24	1455.09	217.40	515.79	665.86	1049.38	3129.38	5310.29 [a]
T250	56.02	269.73	803.29	1462.21	213.71	533.56	658.92	1040.83	3091.81	5103.01 [ab]
T500	55.99	276.23	795.79	1444.65	220.25	519.56	648.86	1042.65	3104.05	4944.92 [b]
T1000	56.37	272.17	804.37	1478.55	215.81	532.19	674.18	1035.43	3177.40	4852.12 [b]
T2000	56.27	270.55	817.36	1479.01	214.29	546.80	661.65	1052.59	3246.72	5103.81 [ab]
Pooled SEM		3.30	10.67	12.91	3.24	9.26	8.45	17.58	63.29	96.32
Treatment		0.6358	0.4113	0.2375	0.5967	0.1370	0.2929	0.9459	0.4040	0.0269
Room	N/A	0.0474	0.0785	0.0340	0.0217	0.1979	0.6792	0.0028	0.0855	0.4021
Experiment		< 0.0001	0.3625	< 0.0001	< 0.0001	0.0002	< 0.0001	0.5179	< 0.0001	0.0015
Treatment × Experiment		0.0504	0.5342	0.1026	0.0385	0.8086	0.3277	0.0847	0.7599	0.4747

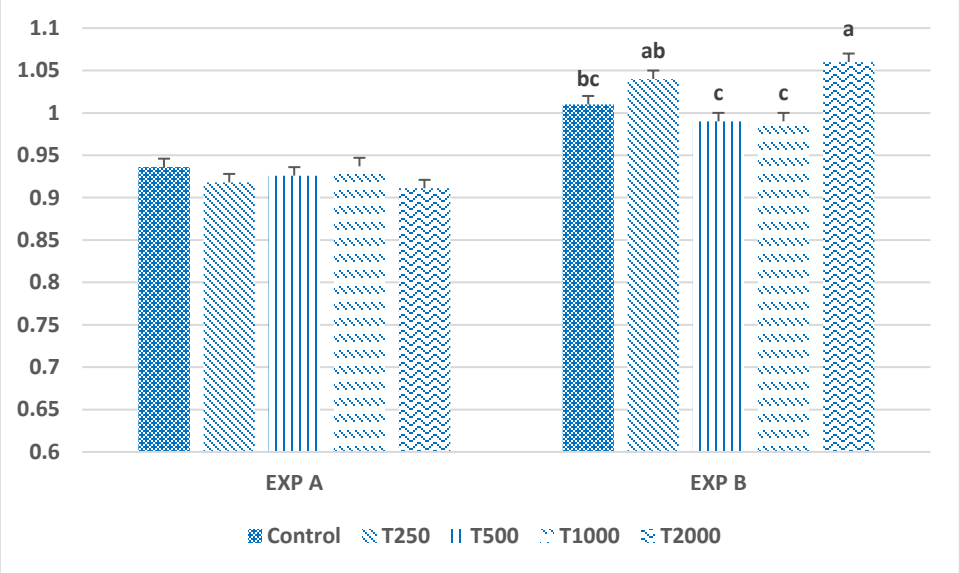
[1] Dietary level of YCW-MOS, 0% (CON), 250 g/ton (T250), 500 g/ton (T500), 1 kg/ton (T1000), and 2 kg/ton (T2000).
[a–b] Means within a column with different superscripts differ ($P \leq 0.05$).

Table 4.3. Effect of YCW-MOS on feed conversion ratio and productivity index from d 7-21 in Pekin ducks

Treatment[1]	Phase FCR			Cumulative FCR		Productivity Index		
	d1 to 7	d7 to 14	d14 to 21	d0 to 14	d1 to 21	d7	d14	d21
CON	0.97	1.23	1.43[b]	1.16	1.28[b]	398.94	486.06	538.33[bc]
T250	0.98	1.17	1.39[ab]	1.12	1.25[ab]	393.50	510.72	559.78[ab]
T500	0.96	1.21	1.46[b]	1.14	1.28[b]	409.83	489.06	527.50[c]
T1000	0.96	1.21	1.32[a]	1.14	1.22[a]	405.61	501.33	569.39[a]
T2000	0.99	1.19	1.37[ab]	1.13	1.24[ab]	396.50	518.39	570.89[a]
SEM	0.0091	0.0181	0.0340	0.0122	0.0162	7.33	10.37	10.91
Treatment	0.1394	0.1427	0.0456	0.2924	0.0198	0.5041	0.1439	0.0179
Room	0.1629	0.2891	0.6106	0.1890	0.3393	0.1137	0.1487	0.3921
Experiment	< 0.0001	< 0.0001	< 0.0001	< 0.0001	< 0.0001	< 0.0001	0.0002	< 0.0001
Treatment × Experiment	0.0006	0.4722	0.4607	0.1317	0.4254	0.0126	0.1804	0.1208

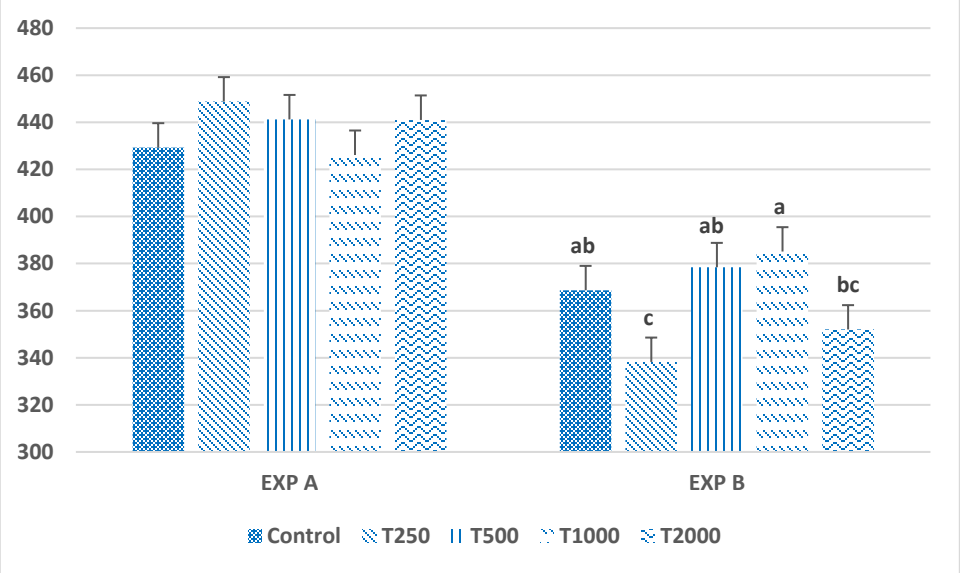
[1] Dietary level of YCW-MOS, 0% (CON), 250 g/ton (T250), 500 g/ton (T500), 1 kg/ton (T1000), and 2 kg/ton (T2000).
[a–b] Means within a column with different superscripts differ ($P \leq 0.05$).

Figure 4.1. Effect of YCW-MOS[1] on pFCR in Pekin ducks at d 1 to 7



[1] Dietary level of YCW-MOS, 0% (CON), 250 g/ton (T250), 500 g/ton (T500), 1 kg/ton (T1000), and 2 kg/ton (T2000).
[a–b] Treatments with different letters within experiment differ ($P \leq 0.05$).

Figure 4.2. Effect of YCW-MOS[1] on PI in Pekin ducks at d 7



[1] **Dietary level of YCW-MOS, 0% (CON), 250 g/ton (T250), 500 g/ton (T500), 1 kg/ton (T1000), and 2 kg/ton (T2000).**
[a–b] **Treatments with different letters within experiment differ ($P \leq 0.05$).**

Table 4.4 presents the amount of manure. Addition of YCW-MOS did not influence the fresh manure amounts. No significant differences in the fresh manure amounts were observed except at d 15. At d 15, T250 had a significantly lower fresh manure amount compared to T1000 and T2000. The groups that consumed more feed, showed a tendency to release more manure. In conclusion, YCW-MOS did not seem to have a significant effect on the manure amount. Waldroup et al. (2003) reported no difference in growth performance between the control group and 1 g/kg of YCW-MOS treated group in d 21 old broiler chickens. Yang et al. (2008) found no significant differences in feed intake, weight gain, and feed conversion efficiency between control, 1 and 2 g/kg of MOS treated groups through 1 to 5 weeks. Effects of YCW-MOS on growth performance was the same even with some pathogenic challenges. Lourenco et al. (2015) also observed no significant difference in weight gains between control and 1 kg/ton of YCW-MOS treated group in d 21 old broiler chickens challenged with *Salmonella enteritidis*. In our study, significant differences were observed in FC, FCR, and PI at d 21 between CON and YCW-MOS treated groups.

In comparison of CON and YCW-MOS treated groups, T1000 had the best effectiveness in FCR at d 21. Therefore, the growth performance results in our study suggest that 1 kg/ton of YCW-MOS could be an ideal dosage for ducklings to derive better growth performance in ducks.

Table 4.4. Effect of YCW-MOS on manure (g) from d 8-21 of Pekin ducks

Treatment[1]	d5	d8	d12	d15	d19	d21
CON	130.27	251.69	503.55	481.56[ab]	627.42	463.58
T250	113.60	212.81	422.53	458.81[a]	586.15	468.05
T500	115.75	213.53	423.39	467.71[ab]	617.43	477.71
T1000	98.40	231.18	472.45	547.02[bc]	664.78	468.82
T2000	119.56	242.00	515.47	562.28[c]	703.24	518.18
SEM	11.64	16.20	36.19	28.64	31.03	26.40
Treatment	0.3337	0.4396	0.2217	0.0311	0.0720	0.4075
Room	0.0622	0.1143	0.0003	0.0004	0.1083	0.0385
Experiment	0.1710	0.3188	0.3235	0.5754	< 0.0001	< 0.0001
Treatment × Experiment	0.0918	0.8219	0.9932	0.8849	0.8601	0.8532

[1] Dietary level of YCW-MOS, 0% (CON), 250 g/ton (T250), 500 g/ton (T500), 1 kg/ton (T1000), and 2 kg/ton (T2000).
[a–b] Means within a column with different superscripts differ ($P \leq 0.05$).

Histomorphological development in the jejunum and ileum

Jejunum and ileum were collected to verify the effects of YCW-MOS on ducklings at d 21. Length (cm), weight (g), organ index, viscosity (cP), and Crypt depth (µm), villi length (µm) and width (µm), size of goblet cell (μm^2) and number of goblet cells of the jejunum and ileum were determined were determined.

Table 4.5 presents results of the intestinal morphology and viscosity. There were no differences in the intestinal length, weight, indices, and viscosity among the groups.

Table 4.6 presents results of the intestinal histomorphology. A significant interaction ($P = 0.0167$) between treatments and experiments was observed in jejunum villi height. T250 and T1000 had significantly greater jejunum villi height compared to T500 and T2000 in experiment B (Figure 4.4). A significant interaction ($P < 0.0001$) between treatments and experiments also was observed in ileum villi height (Figure 4.5). T1000 had significantly greater ileum villi height compared to all other groups in experiment A and T500 had significantly greater ileum villi height compared to CON, T2000, and T250 in experiment B. There were no significant differences in jejunum villi width, but significant interactions ($P = 0.0243$) between treatments and experiments were observed in ileum villi width (Figure 4.6). There was no significant difference in experiment A, but T250 had significantly greater ileum villi width compared to CON, T500, and T2000 in experiment B. A significant interaction ($P = 0.0253$) between treatments and experiments also was observed in jejunum crypt depth. CON had significantly greater jejunum crypt depth compared to T250, T500, and T2000 in experiment A, but in T1000 there was significantly greater jejunum crypt depth compared

to CON, T500, and T2000 in experiment B (Figure 4.7). A significant interaction (P = 0.0173) between treatments and experiments was observed in ileum crypt depth (Figure 4.8). T250 and T1000 had significantly greater ileum crypt depth compared to CON, T500, and T2000 in experiment A and T1000 had significantly greater ileum crypt depth compared to CON, T250, and T500 in experiment B.

These intestinal morphology data indicate that YCW-MOS did not have significant effects on intestinal morphology, which has also been reported in another study. Konca et al. (2009) found no significant difference in intestine indices between CON and 1 kg/ton of YCW-MOS treated groups in 20-week-old turkeys without a pathogenic challenge. However, several other studies observed that YCW-MOS did influence intestinal morphology when YCW-MOS was used with different types of immune challenges. Jahanian et al. (2016) used *Aflatoxin* as a challenge in broiler chickens, and observed significant differences in villi height, width, and depth between non-YCW-MOS treated group and various levels of YCW-MOS treated groups. Santos et al. (2012) used broiler chickens and found their control group had significantly greater jejunum villus height and duodenum, jejunum, and ileum crypt depth compared to their 0.1% of YCW-MOS treated group in d 10 old turkeys when both groups were challenged with *Salmonella enteritidis*. However, the 0.1% YCW-MOS treated group had significantly greater ileum villus height compared to the control group when both groups were challenged with *Salmonella enteritidis*. Another study (Mostafa et al., 2015) used a commercial MOS product with and without *Salmonella*. The authors observed

Table 4.5. Effect of YCW-MOS on intestinal morphology and viscosity from d 21 in Pekin ducks

Treatment[1]	Jejunum				Ileum			
	Length (cm)	Weight (g)	Index	Viscosity (cP)	Length (cm)	Weight (g)	Index	Viscosity (cP)
CON	62.84	21.39	1.48	3.34	69.84	27.29	1.87	4.43
T250	65.53	22.96	1.55	2.95	72.33	27.80	1.90	4.03
T500	63.17	21.56	1.50	2.96	70.29	27.77	1.91	3.68
T1000	63.18	22.50	1.54	3.26	70.84	29.33	1.99	3.84
T2000	64.96	22.22	1.49	3.32	71.23	27.89	1.86	3.90
SEM	1.26	0.58	0.04	0.15	0.82	0.70	0.05	0.22
Treatment	0.4034	0.2630	0.7317	0.1862	0.2554	0.2720	0.3686	0.1582
Room	0.1062	0.2704	0.3873	0.0109	0.2269	0.0018	0.0102	0.1944
Experiment	0.1320	0.0789	0.6896	0.0120	< 0.0001	< 0.0001	0.0002	0.4577
Treatment × Experiment	0.6134	0.8654	0.3624	0.9600	0.6038	0.1680	0.0771	0.4381

[1] Dietary level of YCW-MOS, 0% (CON), 250 g/ton (T250), 500 g/ton (T500), 1 kg/ton (T1000), and 2 kg/ton (T2000).
[a–b] Means within a column with different superscripts differ ($P \leq 0.05$).

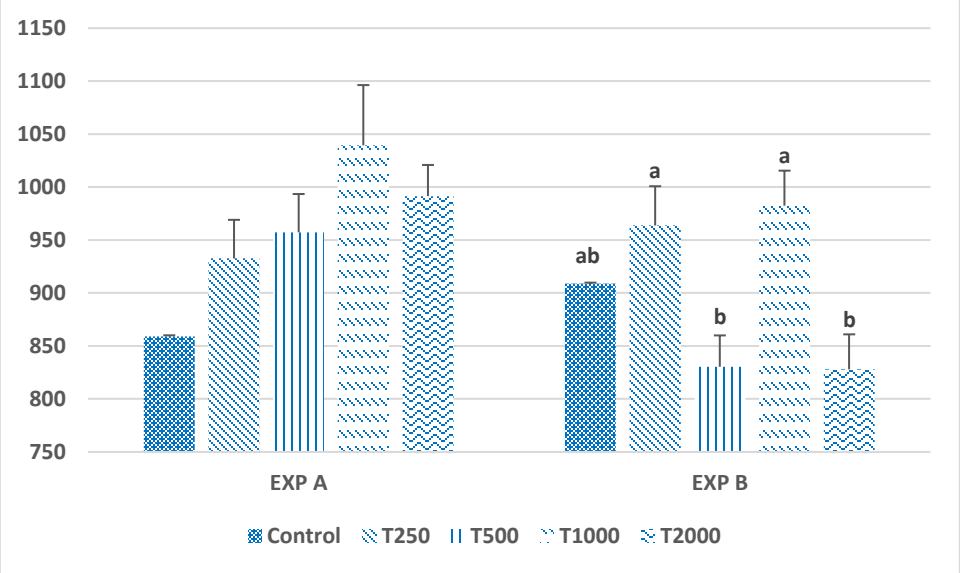
Table 4.6. Effect of YCW-MOS on jejunal and ileal histomorphology from d 21 in Pekin ducks

Treatment[1]	Jejunum					Ileum				
	Crypt Depth (μm)	Villi Height (μm)	Villi Width (μm)	Goblet cell area (μm^2)	Goblet cell numbers	Crypt Depth (μm)	Villi Height (μm)	Villi Width (μm)	Goblet cell area (μm^2)	Goblet cell numbers
CON	167.10	883.93	173.58	30.45^{b}	125.65^{ab}	139.67	676.91	171.89	22.73	80.56^{b}
T250	160.85	949.80	183.22	34.12^{ab}	113.73^{bc}	149.97	676.30	173.18	24.75	84.48^{b}
T500	158.03	868.45	177.80	30.69^{b}	99.71^{c}	132.57	674.26	168.91	22.72	92.46^{b}
T1000	169.44	993.12	182.77	38.35^{a}	140.36^{a}	160.75	720.85	176.89	22.92	116.25^{a}
T2000	148.42	897.40	188.41	32.92^{ab}	120.50^{abc}	140.94	674.03	160.65	24.59	81.44^{b}
SEM	4.92	24.18	6.08	2.11	9.09	4.98	9.98	4.34	1.15	8.38
Treatment	0.0040	0.0064	0.1451	0.0439	0.0350	0.0002	0.0004	0.0178	0.5059	0.0233
Room	0.2846	0.4547	0.0041	0.0012	0.3104	< 0.0001	0.0952	0.1650	0.002	0.0003
Experiment	0.0090	0.0258	0.7993	0.0062	0.5312	< 0.0001	< 0.0001	0.8952	0.3565	0.0042
Treatment × Experiment	0.0253	0.0167	0.4532	0.4525	0.5741	0.0173	< 0.0001	0.0243	0.0132	0.4940

[1] Dietary level of YCW-MOS, 0% (CON), 250 g/ton (T250), 500 g/ton (T500), 1 kg/ton (T1000), and 2 kg/ton (T2000).

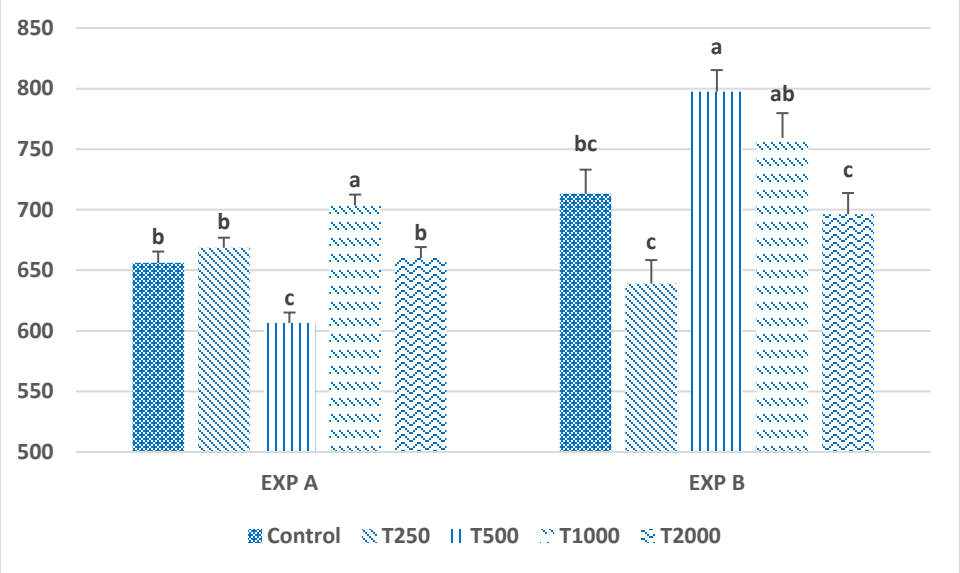
[a–b] Means within a column with different superscripts differ ($P \leq 0.05$).

Figure 4.3. Effect of YCW-MOS[1] on jejunum villi height in Pekin ducks



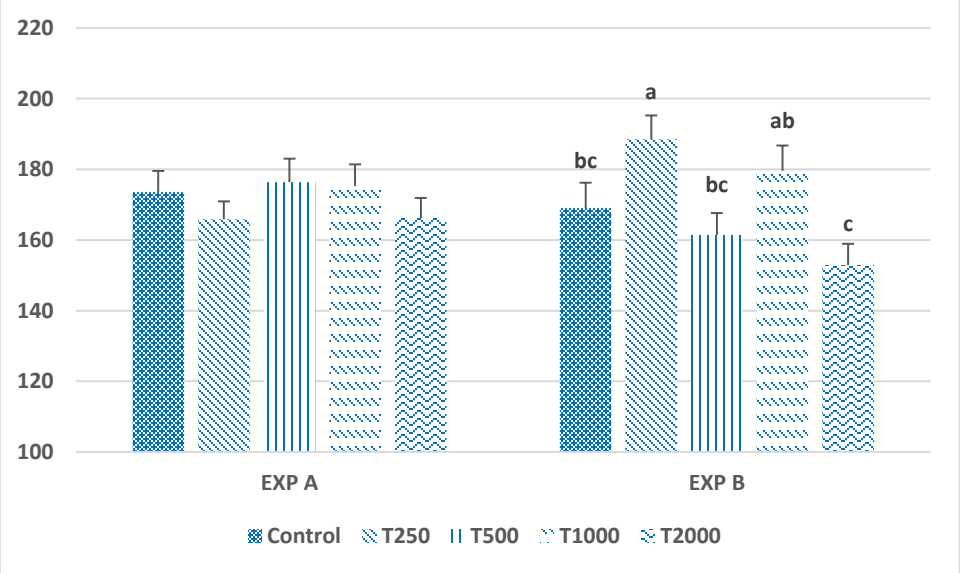
[1] **Dietary level of YCW-MOS, 0% (CON), 250 g/ton (T250), 500 g/ton (T500), 1 kg/ton (T1000), and 2 kg/ton (T2000).**
[a–b] **Treatments with different letters within experiment differ ($P \leq 0.05$).**

Figure 4.4. Effect of YCW-MOS[1] on ileal villi height in Pekin ducks



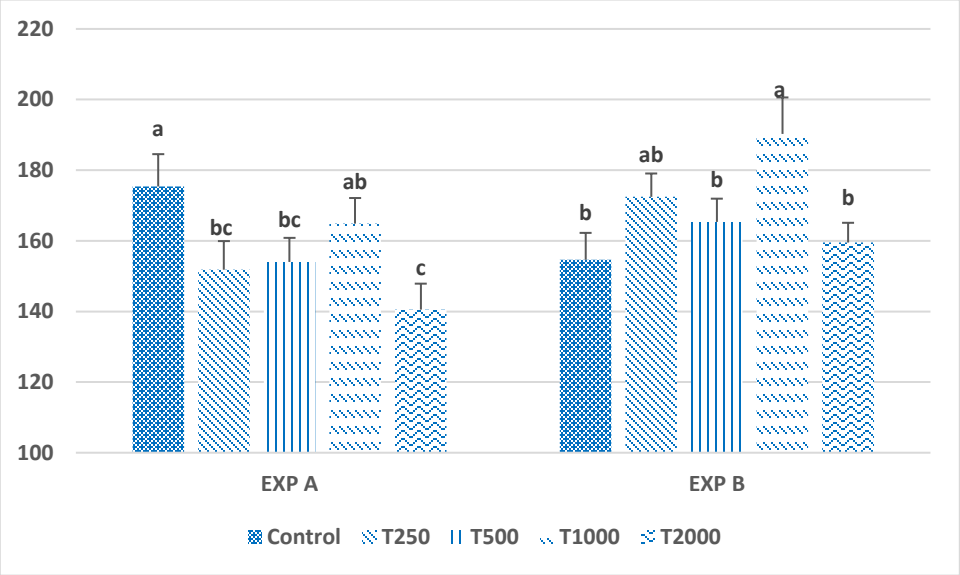
[1] **Dietary level of YCW-MOS, 0% (CON), 250 g/ton (T250), 500 g/ton (T500), 1 kg/ton (T1000), and 2 kg/ton (T2000).**
[a–b] **Treatments with different letters within experiment differ ($P \leq 0.05$).**

Figure 4.5. Effect of YCW-MOS[1] on ileal villi width in Pekin ducks



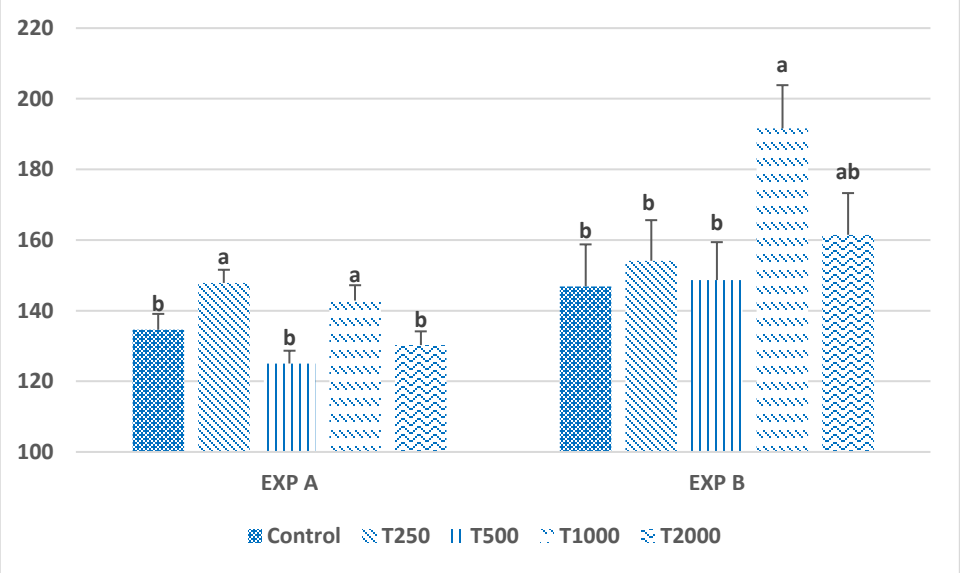
[1] **Dietary level of YCW-MOS, 0% (CON), 250 g/ton (T250), 500 g/ton (T500), 1 kg/ton (T1000), and 2 kg/ton (T2000).**
[a–b] **Treatments with different letters within experiment differ ($P \leq 0.05$).**

Figure 4.6. Effect of YCW-MOS[1] on jejunum crypt depth in Pekin ducks



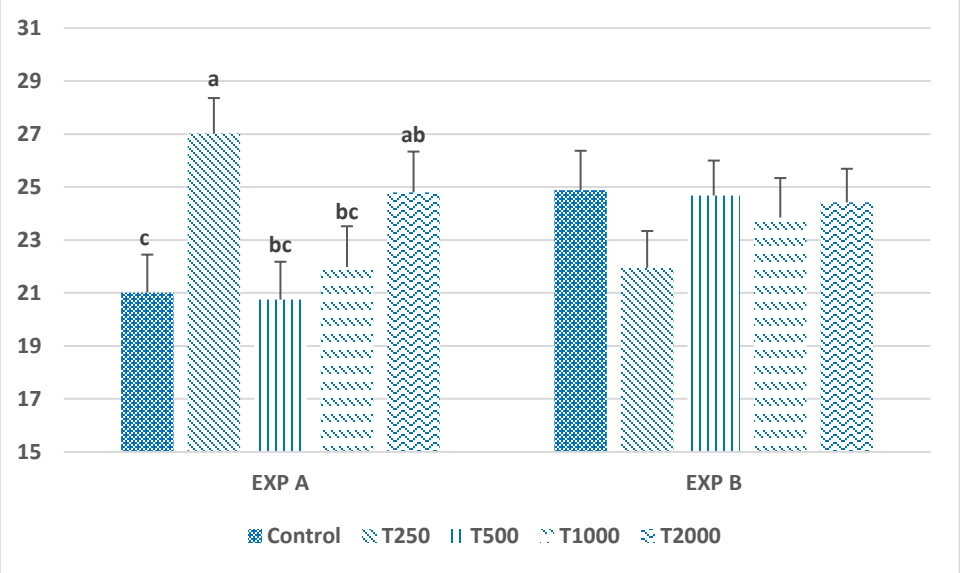
[1] Dietary level of YCW-MOS, 0% (CON), 250 g/ton (T250), 500 g/ton (T500), 1 kg/ton (T1000), and 2 kg/ton (T2000).
[a–b] Treatments with different letters within experiment differ ($P \leq 0.05$).

Figure 4.7. Effect of YCW-MOS[1] on ileal crypt depth in Pekin ducks



[1] **Dietary level of YCW-MOS, 0% (CON), 250 g/ton (T250), 500 g/ton (T500), 1 kg/ton (T1000), and 2 kg/ton (T2000).**
[a–b] **Treatments with different letters within experiment differ ($P \leq 0.05$).**

Figure 4.8. Effect of YCW-MOS[1] on ileal goblet cell area in Pekin ducks



[1] **Dietary level of YCW-MOS, 0% (CON), 250 g/ton (T250), 500 g/ton (T500), 1 kg/ton (T1000), and 2 kg/ton (T2000).**
[a–b] **Treatments with different letters within experiment differ ($P \leq 0.05$).**

that 1 g/kg of MOS treated group had higher jejunum and ileum villus length and lower *Salmonella* population in the ceca. These results indicate that YCW-MOS may only impact intestinal morphology when there is a challenge to stimulate the host immune system. One of the effects of MOS is to guard the intestine from pathogenic attacks. MOS has the ability to protect the host's intestines from pathogenic invasion by binding to pathogens, which are later expelled through the host vent. However, our experiment showed no significant differences in intestinal morphology and histomorphology because no challenge was applied to directly impact the intestinal health. A significant interaction ($P = 0.0132$) between treatments and experiments were observed in ileum goblet cell area (Figure 4.9). T250 and T2000 had significantly greater ileum goblet cell area compared to CON in experiment A. T1000 had significantly greater ($P = 0.0439$) jejunum goblet cell area compared to CON and T500. T1000 had significantly greater ($P = 0.0350$) numbers of goblet cells in jejunum compared to T250 and T500. T1000 also had significantly greater ($P = 0.0233$) numbers of goblet cells in ileum compared to all other groups. Baurhoo et al. (2007) observed comparable results in their chicken study. Birds that consumed MOS had significantly higher jejunum villi height and number of goblet cells per villus compared to the control group. The MOS treated group also had greater numbers of beneficial bacteria (*Lactovacilli, Bifidobacteria*) in the ceca and lower populations of *E. coli* in the litter compared to the control group. Jahanian et al. (2016) also reported equivalent results, which used two different levels of MOS (1 and 2 g/kg). The 2 g/kg treated group showed significantly increased jejunum crypth depth and goblet cell counts. However, Lourenco et al. (2015) found no significant difference in the number of goblet

cells in ileum villi between their control and YCW-MOS treated groups in d 37 old broiler chickens challenged with *Salmonella enteritidis*.

Overall in the present experiments, YCW-MOS had no significant effect on jejunum viscosity and morphology. However, T1000 significantly impacted villi morphologies and numbers of goblet cells in ileum. These histomorphological results correlate with the growth performance results.

Digestibility

The ileal digesta were collected to evaluate the impact of YCW-MOS on digestibility of amino acids in ducklings. Twelve different amino acids (Threonine (Thr), Glycine (Gly), Cysteine (Cys), Valine (Val), Methionine (Met), Isoleucine (Ile), Leucine (Leu), Phenylalanine (Phe), Lysine (Lys), Histidine (His), Arginine (Arg), and Tryptophan (Trp)) were analyzed in this study.

Results of the percentages of the ileal amino acid levels in ducklings are presented in Table 4.7. Briefly, all YCW-MOS treated groups tended to have lower levels of amino acids in ileal digesta compared to CON due to their nutrient absorption improvements by addition of YCW-MOS. A significant difference in amino acid levels in ileal digesta was only observed in Cys. T500 and T1000 had significantly lower ($P \leq 0.0243$) Cys levels in ileal digesta compared to CON. Cys is an important amino acid that plays a role in mucin backbone formation (Horn et al., 2009). Therefore, amino acids absorption has a strong relationship with the histomorphology of the goblet cells.

Results of the ileal amino acid digestibility coefficients in ducklings are presented in Table 4.8. T500 and T1000 had significantly better Cys ($P = 0.0057$) digestibility

compared to CON and T2000. The YCW-MOS treated group had significantly larger and more goblet cells in ileum compared to the non-YCW-MOS treated group. Cys is an important amino acid that plays a role in mucin backbone formation (Horn et al., 2009). Therefore, amino acid absorption has a strong relationship with the histomorphology of the goblet cells. In addition, T500 and T1000 had significantly better Trp (P = 0.0070) digestibility compared to CON. The function of Trp is not still clear to poultry bone, but its metabolism plays a key role in bone composition and formation (Leeson and Summers, 1988). No significant difference was observed in Gly digestibility, but T1000 had numerically better Gly (P = 0.0530) digestibility compared to other groups. Gly is a nonessential amino acid in poultry. However, Gly is required for uric acid synthesizing and for achieving bird maximum growth (Corzo et al., 2004; Corzo et al., 2009). Gly is also required for binding metals. Therefore, Gly not only can be a key factor for a healthy digestive system, but also for mineral absorption. T1000 had significantly better His (P = 0.0380) digestibility compared to CON and T2000. Lake et al. (1980) found that immunoglobulin E mediated mast cell stimulated goblet cell mucin secretion by discharge of histamine in rat duodenum. Therefore, concentration of histamine/histidine in the diet has an effect on stimulation of mucin secretion by gastrointestinal tract goblet cells. Overall, few significant differences in amino acid digestibility were observed between CON and YCW-MOS treated groups. It has been reported through another study that MOS did not significantly impact poultry nutrient digestibility. Yang et al. (2008) observed no significant differences in protein, starch, fat, and soluble and insoluble non-starch polysaccharides digestibility between control and 1 and 2 g/kg of MOS treated groups in

broiler chickens. Also, these results follow trends of other studies of the growth performance and histomorphology in poultry.

Conclusion

In conclusion, T1000 showed the best digestibility among the groups in this study. T1000 showed better amino acid absorption and digestibility for every amino acid numerically and even statistically. Therefore, the 1 kg/ton of YCW-MOS may be the ideal dosage for ducklings to derive better nutrient absorption and amino acid digestibility.

The results from this study confirm that addition of 1 kg/ton of mannan-oligosaccharides in duck feeds positively affects duck growth performance, gut morphology, and digestibility.

Table 4.7. Effect of different levels of YCW-MOS on ileal amino acid levels (%) in Pekin ducks

Treatment[1]	Thr	Gly	Cys	Val	Met	Ile	Leu	Phe	Lys	His	Arg	Trp
CON	0.658	0.711	0.287[b]	0.725	0.178	0.546	0.948	0.550	0.742	0.292	0.537	0.116
T250	0.611	0.658	0.266[a] b	0.669	0.151	0.494	0.864	0.507	0.659	0.268	0.485	0.100
T500	0.607	0.644	0.259[a] b	0.659	0.152	0.488	0.854	0.503	0.655	0.263	0.484	0.099
T1000	0.573	0.621	0.247[a]	0.622	0.137	0.457	0.809	0.443	0.604	0.231	0.415	0.088
T2000	0.619	0.657	0.267[a]	0.669	0.154	0.487	0.863	0.508	0.656	0.268	0.486	0.105
SEM	0.023	0.025	0.008	0.031	0.013	0.026	0.045	0.030	0.046	0.015	0.035	0.007
Treatment	0.1667	0.1318	0.0243	0.2239	0.2862	0.2011	0.2879	0.1633	0.3224	0.0903	0.1909	0.0730
Room	0.0551	0.1909	0.1792	0.1271	0.0921	0.2334	0.1779	0.4687	0.1083	0.4757	0.4481	0.7207
Experiment	0.0126	0.0056	0.0126	0.0055	0.0082	0.0002	0.0003	0.0071	< 0.0001	0.0020	0.0434	0.0330
Treatment × Experiment	0.6824	0.5401	0.4396	0.6785	0.5885	0.5020	0.6215	0.6542	0.6832	0.5313	0.6608	0.4398

[1] Dietary level of YCW-MOS, 0% (CON), 250 g/ton (T250), 500 g/ton (T500), 1 kg/ton (T1000), and 2 kg/ton (T2000).
[a–b] Means within a column with different superscripts differ ($P \leq 0.05$).

Table 4.8. Effect of different levels of YCW-MOS on ileal amino acid digestibility coefficients in Pekin ducks

Treatment[1]	Thr	Gly	Cys	Val	Met	Ile	Leu	Phe	Lys	His	Arg	Trp
CON	69.24	70.41	65.12^{c}	73.78	88.45	78.01	79.44	80.31	77.89	79.68^{b}	84.97	78.62^{c}
T250	71.56	72.99	67.82^{bc}	75.94	90.28	80.28	81.21	81.89	80.96	81.44^{ab}	86.49	82.45^{abc}
T500	72.94	74.53	70.45^{ab}	77.18	90.52	81.16	82.38	82.75	81.39	82.65^{ab}	86.98	83.71^{ab}
T1000	75.26	76.22	72.93^{a}	78.98	91.37	82.98	83.76	85.44	83.59	85.33^{a}	89.41	86.88^{a}
T2000	68.85	70.79	65.68^{c}	73.50	88.38	78.65	79.65	80.19	79.08	79.94^{b}	85.16	80.94^{bc}
SEM	1.635	1.484	1.590	1.537	1.089	1.394	1.300	1.350	1.684	1.344	1.197	1.418
Treatment	0.0528	0.0530	0.0057	0.1023	0.2573	0.1402	0.1540	0.0693	0.2095	0.0380	0.1096	0.0070
Room	0.0368	0.0782	0.0560	0.0530	0.0631	0.0890	0.0729	0.2036	0.0619	0.1731	0.1795	0.3114
Experiment	0.0507	0.0091	0.0078	0.1147	0.0368	0.0051	0.0042	0.0225	0.0052	0.0129	0.0400	< 0.0001
Treatment × Experiment	0.5686	0.8105	0.5166	0.8223	0.4033	0.6288	0.6884	0.6081	0.6539	0.6163	0.6449	0.1823

[1] Dietary level of YCW-MOS, 0% (CON), 250 g/ton (T250), 500 g/ton (T500), 1 kg/ton (T1000), and 2 kg/ton (T2000).

[a–b] Means within a column with different superscripts differ ($P \leq 0.05$).

CHAPTER V

EFFECTS OF A COMMERCIAL BETA-MANNANASE PRODUCT ON GROWTH PERFORMANCE, INTESTINAL HISTOMORPHOLOGY, BONE AND BODY COMPOSITION, AND AMINO ACID DIGESTIBILITY IN PEKIN DUCKS

Introduction

As the indiscriminate use of antibiotics are prohibited in the poultry industry, researchers have specifically focused on the development of innovative alternatives to antibiotic additives in poultry diets to improve growth performance and reduce mortality. Monogastric animals, such as poultry, are not able to digest non-starch polysaccharides (NSPs), hence they often require dietary supplementation of enzymes to break down β (α)-linked NSPs (Klein et al., 2015). Corn and soybean meal are the most common main ingredients for poultry diets that contain β-mannan, which is one kind of NSPs. β-mannan is one of the major materials in polysaccharides that is composed of multiple mannose and glucose units in β-1,4-linkages as the backbone (Liepman et al., 2007), and may also be linked to galactose residues by α-1,6-linkage (Moreira and Filho, 2007). β-mannan is known to increase intestinal viscosity. The increase of intestinal viscosity can lead to reduce nutrient absorption (Lazaro et al., 2003), rate of nutrient passage (Lee et al., 2003), and also modify intestinal morphology (Choct et al., 1999). β-mannanase is an endo-type enzyme and assists in breaking the β-mannan backbone chains. Therefore, if birds ingest the β-mannanase, it increases their growth performance by cleaving the NSPs links, which then improves nutrient digestibility.

Effects of β-mannanase have already been verified through research with chickens. Mussini et al. (2011) used a commercial β-mannanase product in broiler chicken diets. This study used five concentrations of β-mannanase, and the groups treated with β-mannanase showed significantly better amino acid digestibility compared to the control group. Ayoola et al. (2015) used β-mannanase to evaluate effects of β-mannanase on enteric mucosal morphological development and adherent mucin thickness in turkeys. This study found that β-mannanase impacted villi morphology, surface area, and mucin thickness. Even though β-mannanase is one of the most widely used enzymes for poultry, research with β-mannanase in ducks has never been reported in academia. Therefore, this study was conducted with two identical experiments to focus on White Pekin ducks. Our study used five different concentrations of β-mannanase to determine the effects on growth performance, intestinal morphology, bone and body composition, and amino acid digestibility in White Pekin ducks.

Materials and methods

Birds, housing, and diets

For a series of two identical studies (Experiment A and B), White Pekin duck eggs were obtained from a commercial source (Maple Leaf Farms, Leesburg, IN). The eggs were incubated to hatch, and ducklings were screened. Only healthy ducklings were selected at the Texas A&M University Poultry Research, Teaching and Extension Center (TAMUPRC). Vaccine challenges were not applied to the ducklings. A total of 200 birds were allocated into 0.97 × 0.67 × 0.24m size battery cage pens, which allows 0.03m^3/bird at the initial placement. Mixed-sex day-old ducklings were randomly housed with five

birds per battery unit at TAMUPRC. Each treatment was replicated eight times for a total of 40 ducks per treatment. In the experiments, commercial β-mannanase (800,000U/kg) (CTCzyme, CTC Bio Inc., Seoul, Korea) was used. The duck feed formulation was adapted from Zeng et al. (2015) with minor modifications.

The birds were fed corn-soybean meal basal diets (Table 5.1). The experiments consisted of five different treatments: 0% (CON), 0.01% (T01), 0.05% (T05), 0.10% (T10), and 0.20% (T20) of β-mannanase. In both Experiment A and B, starter (d 0-13) and grower (d 14-21) diets were used. The starter and grower diets were pelleted and manufactured at the TAMUPRC feed mill. Each battery cage consisted of one feeder and one water tray and ad libitum supply of feed and water. The lighting was provided for 24 hours from d 0 to 4 and 23 hours from d 5 to 21. The starting room temperature of 30°C was set 48 hours prior to the bird placement. The room temperature was then decreased to 27°C at d 7 and to 23°C at d 14. The birds' circumstances and environment of the housing were monitored daily. There was no replacement of birds during the experiment. These studies were conducted in accordance with an approved animal use protocol from the Institutional Animal Care and Use Committee (AUP: IACUC 2016-0139) of Texas A&M University.

Table 5.1. Experimental diets and nutrient composition

	Starter 1-13 d	Grower 14-21 d
Ingredients (%)		
Corn, yellow grain	42.00	53.70
Soybean meal, dehulled solvent	39.89	27.63
Wheat bran	6.00	6.00
DL Methionine	0.35	0.26
L-lysine	0.07	0.07
Fat, blended A/V	6.74	8.78
Limestone	2.64	1.16
Bio-Phos 16/21 P	1.27	1.35
Salt	0.44	0.44
Trace minerals[1]	0.05	0.05
Vitamins[2]	0.25	0.25
Nutrient Composition		
Crude Protein, %	24.00	19.00
ME, kcal/kg	3038	3298
Crude Fat, %	8.88	11.22
Lysine, %	1.38	1.04
Methionine, %	0.70	0.55
Cysteine, %	0.38	0.32
Tryptophan, %	0.30	0.23
Threonine, %	0.90	0.71
Arginine, %	1.64	1.25
Valine, %	1.10	0.87
Calcium, %	1.33	0.75
Phosphorus, %	0.70	0.68
Sodium, %	0.19	0.19

[1] Trace mineral premix added at this rate yields 149.6 mg manganese, 55.0 mg zinc, 26.4 mg iron, 4.4 mg copper, 1.05 mg iodine, 0.25 mg selenium, a minimum of 6.27 mg calcium, and a maximum of 8.69 mg calcium per kg of diet. The carrier is calcium carbonate, and the premix contains less than 1% mineral oil.

[2] Vitamin premix is added at this rate yields 11,023 IU vitamin A, 3,858 IU vitamin D_3, 46 IU vitamin E, 0.0165 mg B_{12}, 5.845 mg riboflavin, 45.93 mg niacin, 20.21 mg d-pantothenic acid, 477.67 mg choline, 1.47 mg menadione, 1.75 mg folic acid, 7.17 mg peroxidase, 2.94 mg thiamine, 0.55 mg biotin per kg diet. The carrier is ground rice hulls.

Live performance

The body weights were recorded at d 1, 7, 14, and 21. The feed consumption was recorded at d 7, 14, and 21. Productivity index (PI) was calculated by following the formula:

$$\text{PI} = (100 - \text{Mortality}) \times (\frac{\text{BW}}{1000})/\text{Bird Age}/\text{FCR} \times 100$$

The fresh manure was collected from the manure plates at the bottom of each battery cage. The manure weights were recorded at d 7, 10, 14, 17, and 20. The quadratic effect of β-mannanase levels on 21 d BW was analyzed by using Microsoft Excel 2016 (Microsoft Corporation, Redmond, WA).

Collecting samples

At d 21, four randomly chosen birds from each battery unit were euthanized via CO_2 asphyxiation to collect jejunum and ileum samples. Total length of the jejunum and ileum were measured from the first liver portal vein to the Meckel's diverticulum, and from Meckel's diverticulum to the cecal junction, respectively. The jejunum and ileum weights were also recorded to evaluate organ weights and indices. Distal sections of the jejunum and ileum samples were collected from one bird for histology. Digesta from whole sections of the jejunum and ileum were collected for viscosity from one bird. Whole sections of the ileal digesta from two birds were collected to analyze amino acid digestibility.

Viscosity

The samples were evaluated as described by Lee et al. (2003). Digesta from the jejunum and ileum were collected by gentle squeeze. Then, the digesta samples were centrifuged at 4,500 × g for 20 minutes. The supernatants were aliquoted and stored at -

20°C until used. The samples were placed in a viscometer (Brookfield Cone and Plate Viscometer 4 with a CPE-40, Ametek Brookfield, Middleboro, MA) and spindled at 37.8°C. Centipoise (cP) readings were taken after measuring for 20 seconds at 5 rpm.

Histology

The jejunum and ileum samples were rinsed with phosphate buffered saline three times and stored in 70% alcohol (71001-652, VWR International, Radnor, PA) for 24 hours. Then, the samples were transferred into 10% buffered formalin (16004-114, VWR International, Radnor, PA) until fixed. The samples were transferred into 2 × 2 cassettes (97000-390, VWR, Radnor, PA) with 10% buffered formalin. All samples were stained with Alcian Blue pH 2.5 at the Texas A&M University Histopathology/Immunopathology Laboratory. The stained sections were scanned by using NanoZoomer 2.0-HT Digital slide scanner (C9600, Hamamatsu Photonics K.K, Shizuoka Pref., Japan) at the Gastrointestinal Laboratory Department of Small Animal Clinical Sciences at Texas A&M University in order to measure villi height, width, crypt depth, and size and number of goblet cells of the jejunum and ileum using NDP.view2 Viewing Software (Hamamatsu Photonics K.K, Shizuoka Pref., Japan). Ten of jejunum and ileum villi were randomly selected to evaluate villi height, width, and crypt depth. The villus width was measured below half of its height.

Digestibility

An indigestible marker, 5 g/kg of titanium (IV) oxide (248576, Sigma-Aldrich, St. Louis, MO) was added to the grower diet to analyze amino acid digestibility. The collected digesta samples were rinsed with distilled water, and then were freeze-dried (FD4, Thermovac, Island Park, NY). The samples were analyzed by the Agricultural Experiment

Station Chemical Laboratories at the University of Missouri-Columbia. The amino acid digestibility coefficients (AAD) were analyzed as described by Iyayi and Adeola. (2014.) The amino acid digestibility was calculated by following the formula:

$$\text{AAD} = \left\{1 - \left(\frac{\text{Titanium (IV)Oxide (diet)}}{\text{Titanium (IV)Oxide (ieal)}} \times \frac{\text{Amino Acid (diet)}}{\text{Amino Acid (ieal)}}\right)\right\}$$

Body and bone composition analysis

A total of 40 birds (1 bird per unit) was euthanized via CO_2 asphyxiation at d 24 and immediately transferred to the Applied Exercise Science Laboratory at Texas A&M University for Dual-energy X-ray Absorptiometry scanning to evaluate bone mineral density (BMD) and contents (BMC) as well as amounts of lean and fat tissues in the duck bodies. To determine their body and bone compositions, for each scan, five to six randomly selected ducks were scanned twice, dorsal side up. In addition, both left and right tibiae were harvested to determine bone composition and strength. The bone length and weight were determined after bones were defatted with petroleum ether (UN1268, Avantor, Center Valley, PA). The left tibiae were used to determine bone ash. The dried bones were ashed at 600°C for 16 hours (Vulcan 3-1750 NEY Muffle furnace, Thomas Scientific, Swedesboro, NJ). Right tibiae were used to determine bone strength. The bones were sheared midshaft using a crosshead speed of 5.0 mm/min (TA.XT Plus texture analyser, Texture Technologies Corp., South Hamilton, MA).

Statistical analysis

Pooled data from both Experiment A and B were analyzed via a 5 (treatments) × 2 (experiments) factorial analysis of variance using the Standard Least Squares procedure

and completely randomized block design in the JMP Pro® 12.0.1 for Windows (SAS Institute Inc., Cary, NC). The data means were separated using the Least Square Means Differences Student's t-test and deemed significantly different at $P \leq 0.05$.

Results and discussion

Growth performances

To investigate effects of β-mannanase in duckling diets, mortality, average body weight per bird (g), weight gain per bird (g), the cumulative and phase of feed conversion ratio, amount of manure (g), and the productivity index were observed. Three mortalities were observed from Experiment A: one mortality from the CON, one mortality from the T05, and one mortality from the T10. No mortalities were observed from Experiment B. Therefore, β-mannanase did not impact the mortality of ducklings.

Table 5.2 presents results of the body weights (BW), weight gain (WG), and feed consumption (FC). All β-mannanase treated groups had significantly greater BW compared to CON at d 14 ($P < 0.0001$) and at d 21 ($P = 0.0007$), respectively. Treatments T01 and T10 had significantly greater 14d BW than T05. All β-mannanase treated groups had significantly ($P < 0.0001$) more WG compared to CON at d 14. A significant difference in WG was observed between T01, T10, and T20 compared to T05 at d 14. Treatments T05, T10, and T20 had significantly ($P = 0.0105$) more WG compared to CON at d 21. No significant differences were observed in FC. The quadratic dose effect of β-mannanase on the BW of d 21 old of ducklings is presented in Figure 5.1. The model estimated that the ideal dose of β-mannanase was 0.119 %.

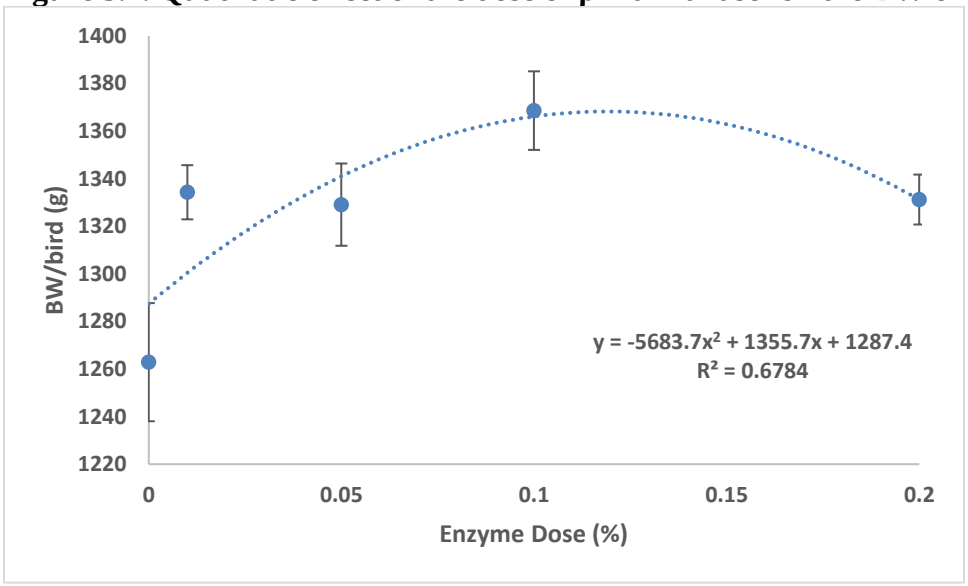
Table 5.3 presents results of the phase feed conversion ratio (pFCR) and cumulative feed conversion ratio (cFCR) and productivity index (PI). All β-mannanase treated groups had significantly improved cFCR compared to CON at d 14 ($P < 0.0001$) and at d 21 ($P = 0.0002$), respectively. All β-mannanase treated groups had significantly greater pFCR compared to CON at d 7 ($P < 0.0001$) and at d 14 ($P = 0.0015$), respectively. All β-mannanase treated groups had significantly better PI compared to CON at d 7 ($P = 0.0009$), at d 14 ($P < 0.0001$), and at d 21 ($P = 0.0003$), respectively. Similar to the other results, a significant difference in PI was observed between T01 and T10 compared to T05 at d 14. When β-mannanase treated groups were compared to the control group, there was no significant effect by addition of β-mannanase supplement on the amount of manure excretion (data not shown). Therefore, β-mannanase did not have a significant impact on the manure amount of ducklings.

Table 5.2. Effect of β-mannanase on body weights per bird (g), weight gain per bird (g), and feed consumption per period (g) from d 1-21 in Pekin ducks

Treatment[1]	Body weight (g)				Weight gain (g)			Feed Consumption (g)		
	d1	d7	d14	d21	d7	d14	d21	d7	d14	d21
CON	57.85	208.59	649.21^{c}	1262.88^{b}	150.74	440.63^{c}	577.59^{b}	177.94	568.06	925.10
T01	57.75	225.25	727.54^{a}	1334.29^{a}	167.50	502.29^{a}	614.91^{ab}	180.85	561.29	935.58
T05	57.98	222.16	691.85^{b}	1329.09^{a}	164.19	469.69^{b}	637.24^{a}	179.54	549.76	930.78
T10	58.00	226.30	722.24^{a}	1368.61^{a}	168.30	495.94^{a}	638.21^{a}	184.90	565.98	952.74
T20	57.65	221.58	719.45^{ab}	1331.23^{a}	163.93	497.88^{a}	647.85^{a}	180.36	561.23	969.91
SEM		4.89	11.55	17.76	4.86	9.28	16.52	4.60	9.64	15.26
Treatment		0.0597	< 0.0001	0.0007	0.0577	< 0.0001	0.0105	0.7537	0.7668	0.2181
Room	N/A	0.0005	0.0219	0.1867	0.0008	0.4353	0.4612	0.0043	0.0955	0.5328
Experiment		0.0027	0.2628	0.0002	< 0.0001	0.8147	0.0010	0.1466	0.7490	< 0.0001
Treatment × Experiment		0.7215	0.5842	0.1944	0.6612	0.6368	0.9424	0.6346	0.3524	0.4974

[1] Dietary level of β-mannanase, 0% (CON), 0.01% (T01), 0.05% (T05), 0.10% (T10), and 0.20% (T20).
[a–c] Means within a column with different superscripts differ ($P \leq 0.05$).

Figure 5.1. Quadratic effect of the dose of β-mannanase[1] on the BW of d 21



[1] Dietary level of β-mannanase, 0% (CON), 0.01% (T01), 0.05% (T05), 0.10% (T10), and 0.20% (T20).

Table 5.3. Effect of β-mannanase on feed conversion ratio and productivity index from d 7-21 in Pekin ducks

Treatment[1]	Phase FCR			Cumulative FCR		Productivity Index		
	d0 to 7	d7 to 14	d14 to 21	d0 to 14	d0 to 21	d7	d14	d21
CON	1.20[b]	1.30[b]	1.53	1.27[b]	1.40[b]	253.81[b]	370.75[c]	434.56[b]
T01	1.09[a]	1.14[a]	1.52	1.12[a]	1.31[a]	299.00[a]	458.19[a]	484.38[a]
T05	1.10[a]	1.19[a]	1.51	1.16[a]	1.32[a]	291.75[a]	432.06[b]	479.06[a]
T10	1.11[a]	1.14[a]	1.52	1.13[a]	1.32[a]	296.06[a]	456.75[a]	489.13[a]
T20	1.11[a]	1.13[a]	1.55	1.12[a]	1.33[a]	289.13[a]	458.94[ab]	476.50 [a]
SEM	0.01	0.03	0.03	0.02	0.01	8.34	10.98	9.47
Treatment	< 0.0001	0.0015	0.8294	< 0.0001	0.0002	0.0009	< 0.0001	0.0003
Room	0.0005	0.0699	0.9981	0.0133	0.1161	0.0005	0.1077	0.2457
Experiment	< 0.0001	0.3510	0.0217	0.1819	0.2290	< 0.0001	0.0513	0.1761
Treatment × Experiment	0.7867	0.9129	0.0816	0.9305	0.5164	0.7370	0.6109	0.2016

[1] Dietary level of β-mannanase, 0% (CON), 0.01% (T01), 0.05% (T05), 0.10% (T10), and 0.20% (T20).
[a–c] Means within a column with different superscripts differ ($P \leq 0.05$).

In this study, β-mannanase treated groups showed significantly better growth performance compared to CON. These trends were also observed in several other studies that used β-mannanase in broiler chickens (Aditya et al., 2014; Ha et al., 2017). Both chicken-based studies also observed that β-mannanase treated groups showed significantly improved growth performance. These results may confirm that β-mannanase can improve growth performance significantly in White Pekin ducks.

Viscosity and histomorphological development in the jejunum and ileum

Two different sections of the small intestine (jejunum and ileum) were collected to examine the effect of β-mannanase on ducklings at d 21. Length (cm), weight (g), organ index, viscosity (cP), crypt depth, villi length and width (μm), and goblet cell size and numbers/villi (μm^2) of the jejunum and ileum were determined (Figure 5.2).

Table 5.4 presents results of the intestinal morphology and viscosity. There were no significant differences in the jejunum length (P = 0.4918) and index (P= 0.7953). No significant differences were observed among the groups in ileum index (P = 0.5901). However, significant interactions between treatments and experiments were observed in both jejunum (P = 0.0093) and ileum (P = 0.0362) weight. Jejunum weights of all β-mannanase treated groups were significantly greater than CON in Experiment A, but there were no significant differences among the groups in Experiment B (Figure 5.3). Also, T01 and T10 had significantly greater ileum weight compared to CON in Experiment A, but there were no significant differences among the groups in Experiment B (Figure 5.4). The jejunum and ileum weight results in both Experiment A and B had no significant differences among the groups. All β-mannanase treated groups had significantly (P =

0.0051) longer ileum length compared to CON (Table 5.4). T01 and T05 had significantly (P = 0.0433) lower ileal viscosity compared to CON. However, there was no significant difference among the groups in jejunal viscosity results (P = 0.4959). Mehri et al. (2010) observed equivalent intestinal viscosity results where β-mannanase treated groups had statistically lower ileal viscosity than control group. These results demonstrate that β-mannanase affected the intestinal morphology and viscosity of ducklings significantly.

Table 5.4. Effect of β-mannanase on intestinal morphology and viscosity in White Pekin ducks

Treatment[1]	Jejunum				Ileum			
	Length (cm)	Weight (g)	Index	Viscosity (cP)	Length (cm)	Weight (g)	Index	Viscosity (cP)
CON	64.31	21.26	1.67	1.96	65.13[b]	26.70	2.09	3.05[a]
T01	66.46	22.22	1.65	2.35	69.02[a]	28.29	2.17	2.47[b]
T05	66.04	21.51	1.62	2.04	68.35[a]	28.58	2.16	2.31[b]
T10	66.21	22.43	1.66	2.05	69.23[a]	28.68	2.06	2.69[ab]
T20	65.06	21.96	1.64	2.02	67.81[a]	27.79	2.09	2.64[ab]
SEM	1.09	0.56	0.05	0.17	0.91	0.66	0.06	0.17
Treatment	0.4918	0.4051	0.7953	0.4959	0.0051	0.1587	0.5901	0.0433
Room	0.0004	0.0245	0.1629	0.7219	0.0928	0.0056	0.0482	0.0367
Experiment	0.9984	0.6418	0.0101	0.1646	0.0741	< 0.0001	< 0.0001	< 0.0001
Treatment × Experiment	0.3825	0.0093	0.1459	0.1084	0.3322	0.0362	0.5338	0.7491

[1] Dietary level of β-mannanase, 0% (CON), 0.01% (T01), 0.05% (T05), 0.10% (T10), and 0.20% (T20).
[a–c] Means within a column with different superscripts differ ($P \leq 0.05$).

Figure 5.2. Section of intestinal tissue of White Pekin duck.

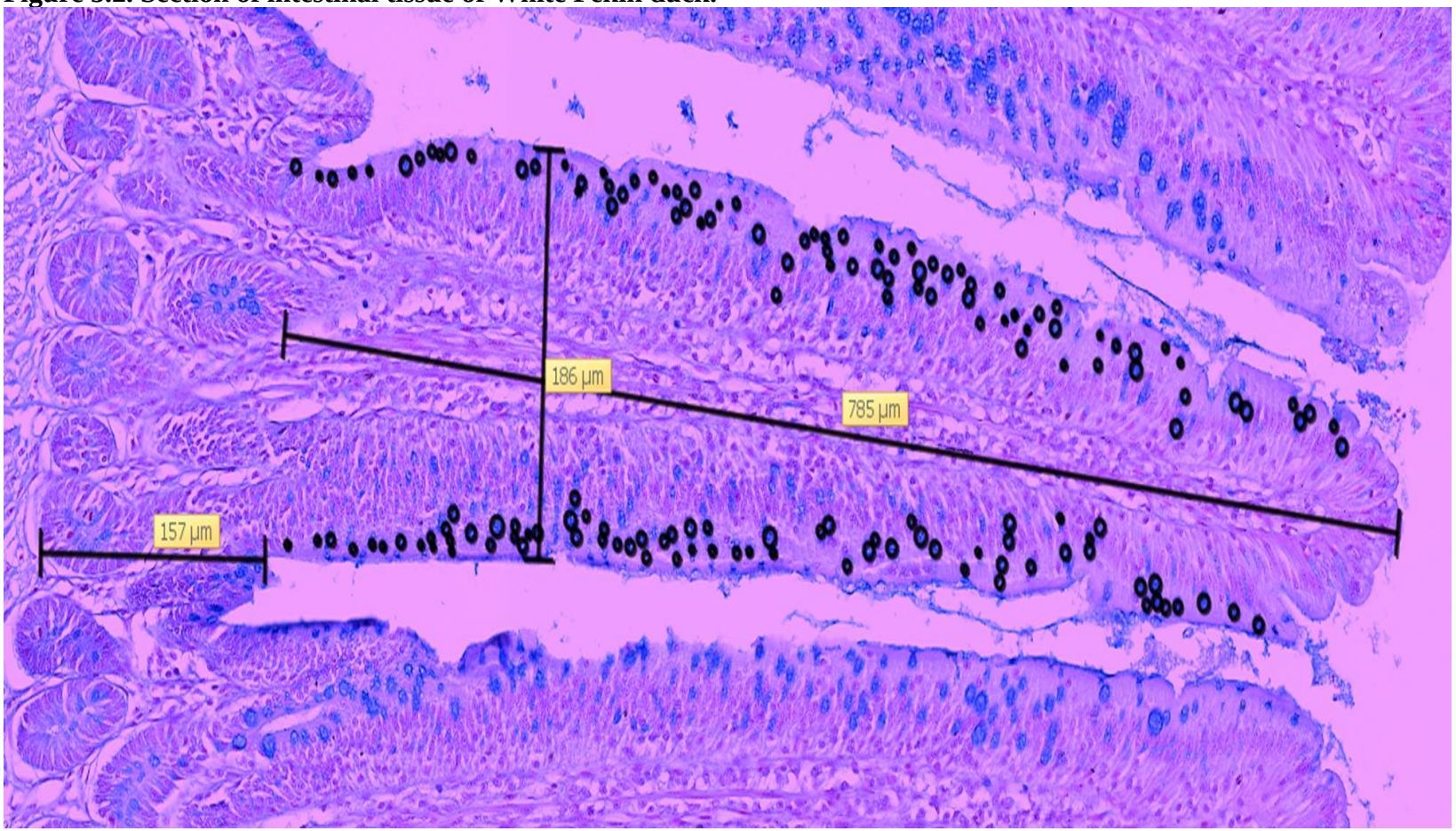
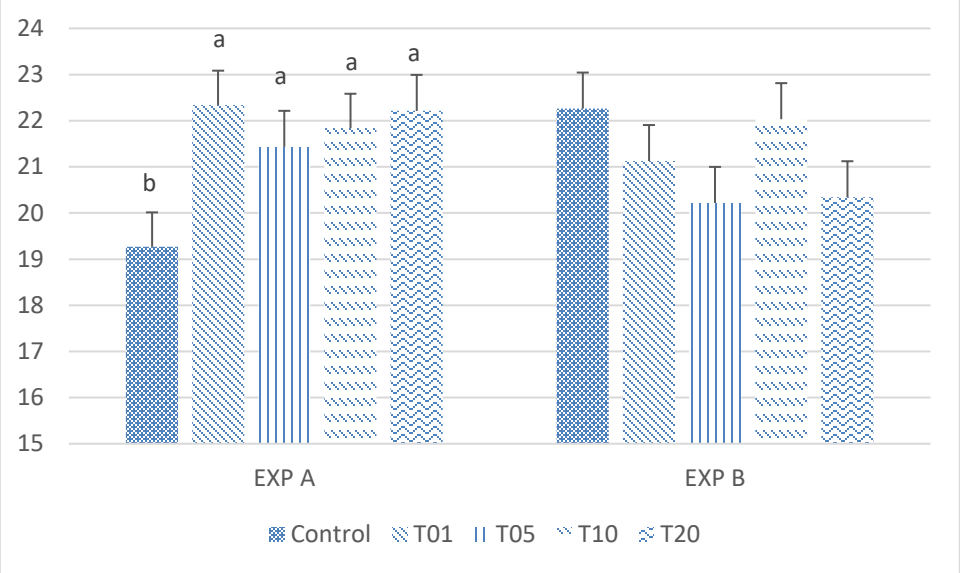
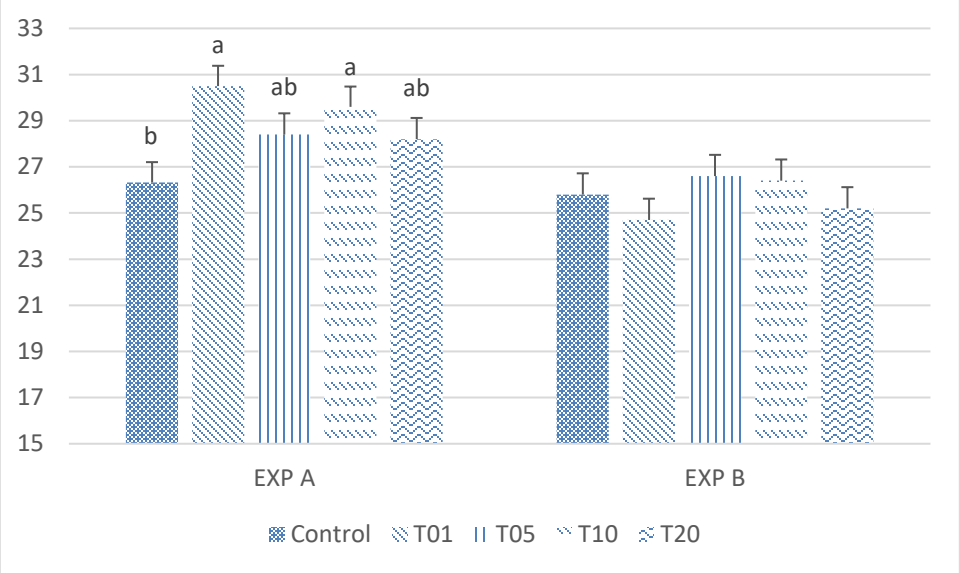


Figure 5.3. Effect of β-mannanase[1] on jejunum weight in Pekin ducks



[1] **Dietary level of β-mannanase, 0% (CON), 0.01% (T01), 0.05% (T05), 0.10% (T10), and 0.20% (T20).**
[a–b] **Treatments with different letters within experiment differ ($P \leq 0.05$).**

Figure 5.4. Effect of β-mannanase[1] on ileum weight in Pekin ducks



[1] Dietary level of β-mannanase, 0% (CON), 0.01% (T01), 0.05% (T05), 0.10% (T10), and 0.20% (T20).
[a–b] Treatments with different letters within experiment differ ($P \leq 0.05$).

Table 5.5 presents results of intestinal histomorphology. There was no significant difference in jejunum crypt depth (P = 0.5382). Significant interactions between treatments and experiments were observed in jejunum villi height (P = 0.0142) and width (P = 0.0250). CON had significantly greater jejunum villi height compared to T05 in Experiment A; and T05 had significantly greater jejunum villi height compared to T01 in Experiment B (Figure 5.5). CON, T01, T05, and T10 treated groups had significantly greater jejunum villi width compared to T20 in Experiment A; and T20 had significantly greater jejunum villi width compared to CON in Experiment B (Figure 5.6) Significant differences were observed in ileum villi height and width, and crypt depth (Table 5.5). T10 had significantly (P = 0.0069) greater ileal villi height compared to CON, T01, and T20. Treatments T05, T10, and T20 had significantly (P = 0.0095) greater ileum villi width compared to CON. T05, T10, and T20 had significantly ($P < 0.0001$) greater ileum crypt depth compared to CON and T01. β-mannanase had no significant effect on jejunum morphology development. However, β-mannanase did affect ileum morphology development. Especially, T10 showed significant impacts on ileum villi width and crypt depth. The intestinal morphology trends with β-mannanase that impacted intestinal morphology have also been observed in another study with broiler chickens. Saenphoom et al. (2013) observed no differences in jejunum and ileum villi height and crypt depth of broiler chickens between mannanase treated and non-mannanase treated groups. The authors found significant differences only in duodenal crypt depth among the treatments. Another study, Mehri et al. (2010) also observed similar histomorphology results with broiler chickens. The authors observed that β-mannanase treated groups had significantly

greater jejunal villi height, crypt depth, and ileal crypt depth. A significant difference was not observed in ileum goblet cell size ($P = 0.1541$), but a significant ($P = 0.0076$) treatment by experiment interaction was observed in jejunum goblet cell size (Table 5.5). T20 had significantly greater jejunum goblet cell size compared to T01 and T05 in Experiment A and T05 and T20 had significantly greater jejunum goblet cell size compared to CON in Experiment B (Figure 5.7). Significant differences were not observed among the groups in the population of goblet cells in the jejunum ($P = 0.1041$). T10 had significantly ($P = 0.0006$) greater number of ileum goblet cells compared to all other groups. T05 and T20 also had significantly greater numbers of ileum goblet cells compared to CON, but there was no significant difference between CON and T01. β-mannanase had no effect on ileum goblet cell size, but effected ileum goblet cell population. Therefore, the population of goblet cells is more responsive to the treatments than the size of goblet cells. Unlike our study, another study (Mehri et al. 2010) observed contradictory results where the β-mannanase treated group had significantly lower populations of goblet cells than the control group in both jejunum and ileum in broiler chickens. According to our results, since T10 had the highest population of goblet cells, this result verified again that 0.1 % of β-mannanase is close to the most ideal β-mannanase level (0.119 %) based on the body weight at d 21 (Figure 5.1).

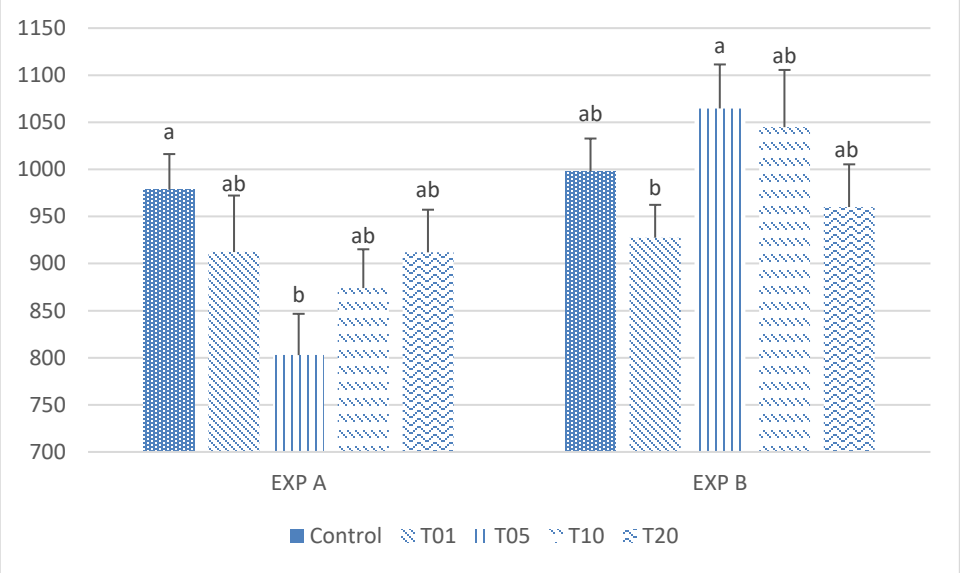
Overall, β-mannanase in these experiments had significant impacts on ileum morphology and viscosity, but not on jejunum morphology and viscosity. The histomorphological results are consistent with growth performance. In conclusion, 0.1%

Table 5.5. Effect of β-mannanase on intestinal histomorphology in Pekin ducks

Treatment[1]	Jejunum					Ileum				
	Crypt Depth (μm)	Villi Height (μm)	Villi Width (μm)	Goblet cell area (μm^2)	Goblet cell numbers	Crypt Depth (μm)	Villi Height (μm)	Villi Width (μm)	Goblet cell area (μm^2)	Goblet cell numbers
CON	168.21	1008.39	204.13	28.33^{b}	119.30	144.88^{b}	652.72^{b}	175.82^{b}	23.25	85.86^{c}
T01	168.44	950.61	218.48	28.01^{b}	126.13	149.63^{b}	668.02^{b}	186.26^{ab}	21.05	104.85^{bc}
T05	177.76	976.18	208.67	30.83^{ab}	121.25	158.06^{a}	674.00^{ab}	201.74^{a}	19.42	108.27^{b}
T10	167.55	954.15	224.91	29.03^{ab}	147.60	161.50^{a}	717.25^{a}	198.10^{a}	24.66	130.43^{a}
T20	164.62	976.06	201.71	34.24^{a}	125.93	157.45^{a}	644.36^{b}	193.29^{a}	22.58	108.69^{b}
SEM	5.89	33.05	8.17	2.12	11.77	3.85	13.26	6.02	1.36	8.63
Treatment	0.5382	0.4666	0.2723	0.0404	0.1041	< 0.0001	0.0069	0.0095	0.1541	0.0006
Room	0.1485	0.0002	0.2614	0.0078	0.0214	< 0.0001	0.0344	0.0342	0.0156	0.3155
Experiment	0.2600	0.0005	0.0936	0.9714	0.4576	0.0648	< 0.0001	0.7372	0.0003	0.0020
Treatment × Experiment	0.0580	0.0142	0.0250	0.0076	0.6843	0.1522	0.0578	0.2257	0.1652	0.3552

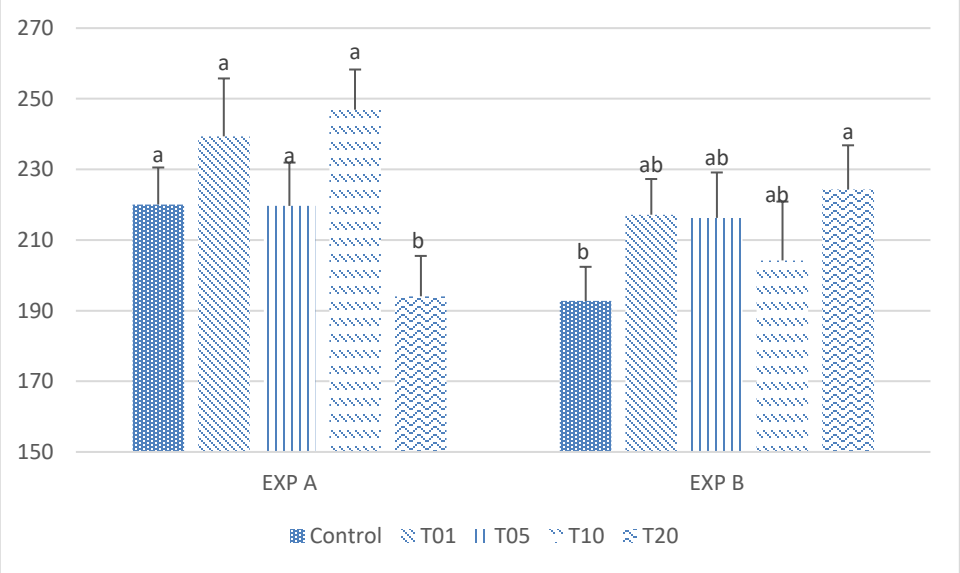
[1] Dietary level of β-mannanase, 0% (CON), 0.01% (T01), 0.05% (T05), 0.10% (T10), and 0.20% (T20).
[a–c] Means within a column with different superscripts differ ($P \leq 0.05$).

Figure 5.5. Effect of β-mannanase[1] on jejunum villi height in Pekin ducks



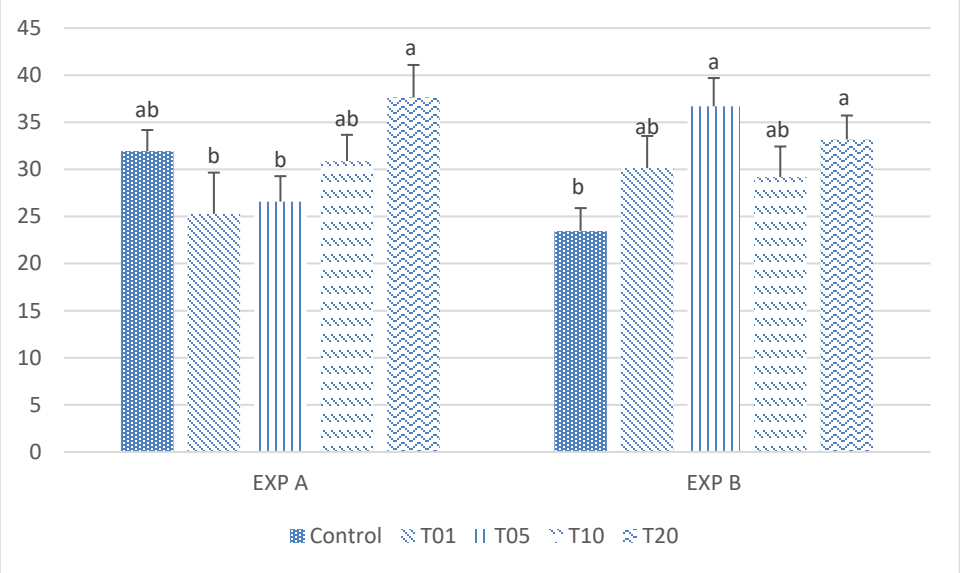
[1] **Dietary level of β-mannanase, 0% (CON), 0.01% (T01), 0.05% (T05), 0.10% (T10), and 0.20% (T20).**
[a–b] **Treatments with different letters within experiment differ ($P \leq 0.05$).**

Figure 5.6. Effect of β-mannanase[1] on jejunum villi width in Pekin ducks



[1] **Dietary level of β-mannanase, 0% (CON), 0.01% (T01), 0.05% (T05), 0.10% (T10), and 0.20% (T20).**
[a–b] **Treatments with different letters within experiment differ ($P \leq 0.05$).**

Figure 5.7. Effect of β-mannanase[1] on jejunum goblet cell area in Pekin ducks



[1] **Dietary level of β-mannanase, 0% (CON), 0.01% (T01), 0.05% (T05), 0.10% (T10), and 0.20% (T20).**
[a–b] **Treatments with different letters within experiment differ ($P \leq 0.05$).**

of β-mannanase appears to be the ideal level to induce optimal intestinal morphology and viscosity.

Digestibility

The ileal digesta were collected to verify effects of β-mannanase on digestibility of amino acids in ducklings. Twelve different amino acids (Threonine (Thr), Glycine (Gly), Cysteine (Cys), Valine (Val), Methionine (Met), Isoleucine (Ile), Leucine (Leu), Phenylalanine (Phe), Lysine (Lys), Histidine (His), Arginine (Arg), and Tryptophan (Trp)) were analyzed in this study.

Results of the ileal amino acid digestibility coefficients in ducklings are presented in Table 5.6. All β-mannanase treated groups had significantly greater ileal Thr ($P < 0.0001$), Gly ($P < 0.0001$), Cys ($P < 0.0001$), Val ($P < 0.0001$), Met ($P < 0.0001$), Ile ($P < 0.0001$), Leu ($P < 0.0001$), Phe ($P < 0.0001$), Lys ($P < 0.0001$), His ($P < 0.0001$), and Arg ($P < 0.0001$) digestibility compared to CON. These results had similarities with another study that used broiler chickens. Mussini et al. (2011) used 0%, 0.025%, 0.05%, and 0.1% of β-mannanase in broiler chicken diets. The authors reported that β-mannanase treated groups had significantly greater ileal amino acid digestibility compared to the control group. The authors also observed that ileal amino acid digestibility was significantly increased with increasing β-mannanase concentration. However, there were no significant differences among the β-mannanase treated groups in our study, except in Trp digestibility. T10 had significantly greater ($P < 0.0001$) ileal Trp digestibility compared to CON and T20.

Table 5.6. Effect of different levels of β-mannanase on ileal amino acid digestibility coefficients in Pekin ducks

Treatment[1]	Thr	Gly	Cys	Val	Met	Ile	Leu	Phe	Lys	His	Arg	Trp
CON	49.26^{b}	55.42^{b}	45.41^{b}	57.47^{b}	80.59^{b}	63.03^{b}	66.06^{b}	65.14^{b}	63.48^{b}	66.36^{b}	73.27^{b}	66.99^{c}
T01	71.56^{a}	73.10^{a}	69.59^{a}	76.16^{a}	87.90^{a}	79.10^{a}	80.44^{a}	80.59^{a}	78.16^{a}	81.28^{a}	84.59^{a}	81.51^{ab}
T05	72.27^{a}	73.13^{a}	71.42^{a}	76.79^{a}	90.15^{a}	79.45^{a}	80.82^{a}	80.80^{a}	79.18^{a}	81.37^{a}	85.16^{a}	81.80^{ab}
T10	75.26^{a}	76.47^{a}	74.01^{a}	79.21^{a}	89.87^{a}	81.85^{a}	82.91^{a}	82.98^{a}	81.14^{a}	83.51^{a}	86.36^{a}	85.25^{a}
T20	72.46^{a}	74.06^{a}	71.26^{a}	76.76^{a}	88.15^{a}	79.45^{a}	80.65^{a}	80.62^{a}	78.20^{a}	81.23^{a}	84.37^{a}	79.70^{b}
SEM	1.716	1.526	1.602	1.586	1.099	1.472	1.352	1.323	1.800	1.276	1.214	1.633
Treatment	< 0.0001	< 0.0001	< 0.0001	< 0.0001	< 0.0001	< 0.0001	< 0.0001	< 0.0001	< 0.0001	< 0.0001	< 0.0001	< 0.0001
Room	0.0447	0.0424	0.1079	0.0672	0.1180	0.0832	0.0914	0.0902	0.0931	0.0999	0.1151	0.1634
Experiment	0.2579	0.2559	0.0581	0.0600	0.2885	0.1809	0.0686	0.2810	0.6518	0.2202	0.2065	0.0008
Treatment × Experiment	0.2898	0.2204	0.8170	0.2094	0.3574	0.2727	0.3440	0.2239	0.2727	0.4247	0.3446	0.4868

[1] Dietary level of β-mannanase, 0% (CON), 0.01% (T01), 0.05% (T05), 0.10% (T10), and 0.20% (T20).

[a–c] Means within a column with different superscripts differ ($P \leq 0.05$).

His and Thr play important roles in mucin secretion. Lake et al. (1980) reported that goblet cell mucin secretion function was stimulated by discharge of histamine from immunoglobulin E mediated mast cell. Especially, threonine has functions such that synthesis of the mucin protein and protein phosphorylation and *O*-linked glycosylation in the intestine (Mao et al., 2011). Horn et al. (2009) performed a threonine deficiency experiment on White Pekin ducks to find a correlation between mucin secretion and threonine. The authors reported that mucin secretion was increased by increasing the threonine concentration in duck diets. Goblet cell density and expression of mucin gene (MUC2) mRNA abundance were also increased as threonine increased. However, the authors did not find a correlation between threonine deficiency and mucin secretion in broiler chickens. Trp and Cys are also counted as important materials that are required for mucin backbone formation and synthesizing mucin protein, respectively (Horn et al., 2009; Wu, 2013). In our amino acid digestibility results, all β-mannanase treated groups had greater ileal His, Thr, and Cys digestibility than CON. T10 had significant improvement in Trp digestibility compared to CON and T20. Therefore, this result verified that T10 has the largest number of ileal goblet cells and demonstrated that there is a strong relationship between amino acid digestibility and goblet cell population.

In conclusion, although mucin layer thickness was not evaluated in this experiment, our histomorphology results showed that T10 had significantly greater ileal goblet cell population compared to all other groups. Our overall histomorphology results showed that T10 had the healthiest small intestine.

Body and bone composition

Results of the body and bone compositions are presented in Table 5.7. No significant differences were observed in BMD (P = 0.5096), BMC (P = 0.9454), bone ash (P = 0.0674), bone length (P = 0.8973) bone weight (P = 0.3017), and the amount of lean tissue (P = 0.2565). However, significant differences in bone strength and amount of fat tissue in duck bodies were observed. T05 had significantly (P = 0.0331) greater bone strength compared to CON and T20. T10 had significantly (P = 0.0189) lower fat tissue compared to CON and T20. These results indicated that β-mannanase impacted the bone strength and the percentage of body fat of the ducklings.

These results are consistent with the result of significantly increased amino acid digestibility. For example, Gly can be an important factor for uric acid synthesizing to achieve maximum growth of birds (Corzo et al., 2004; Corzo et al., 2009). Gly also forms chelates with metals (Ashmead, 1993). Therefore, Gly not only maintains a healthy intestine, but also helps to absorb minerals. In conclusion, β-mannanase affects body and bone composition of White Pekin ducks.

Conclusion

These results confirm that the addition of β-mannanase in the feed of ducks positively impacted the growth performance, gut morphology, digestibility, and body and bone composition of White Pekin ducks.

Table 5.7. Effect of β-mannanase on bone and body composition in Pekin ducks

Treatment[1]	BMD[1] (g/cm^2)	BMC[2] (g)	Lean Tissue (lbs)	Fat Tissue (%)	Bone Ash (%)	Bone Length (cm)	Bone Weight (g)	Bone Strength
T01	0.1437	18.5958	2.5868	12.8667[a]	50.5004	8.5417	2.9883	17.7685[b]
T05	0.1444	19.3065	2.7523	11.7226[ab]	49.7170	8.6438	3.2586	19.1263[ab]
T10	0.1427	19.2069	2.6319	11.5276[ab]	51.6904	8.6400	3.3079	22.3183[a]
T20	0.1396	19.3394	2.7006	11.1152[b]	49.6240	8.6067	3.1954	19.2159[ab]
CON	0.1435	19.1613	2.6407	12.2903[a]	49.5022	8.6938	3.1677	16.6195[b]
SEM	0.0002	0.5000	0.0527	0.4332	0.6814	0.0846	0.0921	1.3313
Treatment	0.5096	0.9454	0.2565	0.0189	0.0674	0.8973	0.3017	0.0331
Room	0.0607	0.0014	0.0199	0.0490	0.1099	0.1964	0.0008	0.9223
Experiment	0.9939	0.6433	0.6948	0.3071	< 0.0001	0.9004	0.0014	< 0.0001
Treatment × Experiment	0.7544	0.1835	0.1166	0.0850	0.2656	0.4978	0.4476	0.1381

[1] Dietary level of β-mannanase, 0% (CON), 0.01% (T01), 0.05% (T05), 0.10% (T10), and 0.20% (T20).
[a–c] Means within a column with different superscripts differ ($P \leq 0.05$).
[1]BMD: Bone Mineral Density
[2]BMC: Bone Mineral Contents

CHAPTER VI

EFFECTS OF A COMMERCIAL BETA-MANNANASE PRODUCT ON THE CHOLESTEROL LEVEL OF BLOOD SERUM, INTESTINAL PH AND VISCOSITY, AND DIGESTA PASSAGE RATE OF WHITE PEKIN DUCKS

Introduction

Non-polysaccharides (NSPs) are naturally occurring components in plant feedstuffs that are known to trigger several adverse effects on poultry as shown through many studies (Mehri et al., 2010; Saenphoom et al., 2013; Mussini et al., 2011), such as increasing gut viscosity (Lee et al., 2003). Utilization of an enzyme in the diet may help increase nutritive benefits from plant feedstuffs if the enzyme hydrolyzes substrates such as NSPs. Previous research (Park et al., 2017a; Park et al., 2017b) has demonstrated that β-mannanase impacts live performance, morphologies of small intestines, gastrointestinal viscosity, and bone and body composition. The digesta viscosity results from these previous studies indicate that enzyme supplementation of corn-soybean meal affects the viscosity and the absorption of nutrients adversely in ducklings. However, these experiments evaluated only the improvement in viscosity by β-mannanase supplementation. They did not evaluate other changes that could cause increased viscosity. Increased digesta viscosity can increase digesta passage rate (Johansen et al., 1996). This slowing down or stagnation of digesta passage rate in the gastrointestinal tract results in a reduction in oxygen levels due to microbial fermentation (Acetic acid formation) (Choct et al., 1996). As anaerobic bacteria population increases in the gut (Choct, 1997), the pH of the digesta will also be decreased because of increased toxin emissions from the bacteria

(Wood and Serfaty-Lacrosniere., 1992). Additionally, cholesterol levels in the blood will decrease due to lower nutrient digestion, absorption and binding of bile salts in the gut (Moundras et al., 1997). These effects not only impact live performance, but ultimately increase the probability of pathogen invasion (Sinha et al., 2011).

Guar seed consisted of endosperm, germ, and hull and guar meal is the mixture of a ratio of 1:3 of germ and hull (Janampet et al., 2016). Guar meal can be an alternative ingredient in poultry diets due to its high protein content (Nagpal et al., 1971) and low price (Gutierrez et al., 2007). These advantages would be useful for countries that depends on importing grains for livestock. For example, South Korea imports more than 90 % of its grain in order to produce feed for livestock. However, guar meal induces more deleterious impacts on the poultry intestine than corn-soybean based feed because residual guar gum in the meal contains approximately 100 g/kg of NSPs (Fillery-Travis et al., 1997).

Several studies have established that guar meal inclusion in broiler diets decreases growth rate (Conner 2002; Lee et al., 2003). In the present study, 10% guar hull fraction was included in the diets to maximize the negative impact of NSPs. Therefore, the objective of this study was to evaluate whether duck diets with 10% guar meal and β-mannanase could support performance equal to that of corn-soybean meal diets. In this study, White Pekin ducks were used to evaluate the effects of the β-mannanase through d 0 to d 21. This study tested and analyzed live performance, pH of the digestive tract, cholesterol level in blood, and feed passage rate.

Materials and methods

Birds, housing, and diets

The experiment was a factorial arrangement of 2 levels of guar (0% and 10%) and 2 levels of β-mannanase (0% and 0.10%). Treatment descriptions are as follows: Control (CON) diet 0% guar and 0% β-mannanase, the 10% guar and 0% β-mannanase diet (GUAR), the 0% guar and 0.10% β-mannanase diet (ENZ), and the 10% guar and 0.10% β-mannanase diet (BOTH). The diets are described in Table 1. White Pekin duck eggs were obtained from a commercial company, Maple Leaf Farms (Leesburg, IN). Eggs were incubated and hatched at the Texas A&M University Poultry Research, Teaching and Extension Center (TAMUPRC). Only healthy ducklings were selected, and vaccine challenges were given to the ducklings. Mixed-sex day-old ducklings were randomly housed in battery cages 0.97 × 0.67 × 0.24 m (six birds per cage), space per bird was approximately 0.03 m^3/bird at initial placement. A total of 96 birds were allocated to the battery cage pens, each treatment was replicated four times for a total of 24 ducks per treatment. In this experiment, starter diet was provided from d 1 through d 21. All diets were pelleted and manufactured at the TAMUPRC feed mill. Each pen was provided an *ad libitum* supply of feed and water. There was one feeder and two water trays in each battery cage. Lighting was provided 24 hours during the first four days; then light was provided 23 hours per day until d 20. At d 21, 22 hours of lighting was provided. Room temperature was set to 30 °C 48 hours before placing of the birds. The room temperature was decreased 3 °C at d 7 to reach 27 °C and was decreased 4 °C at d 14 to reach 23 °C. During the experiment, no birds were replaced due to mortality. The birds' health and

room environment were monitored daily. These studies were conducted in accordance with an approved animal use protocol (IACUC 2016-0139) from the Institutional Animal Care and Use Committee of Texas A&M University.

Growth performance

Body weights (BW) were recorded at d 1, 7, 14 and 20. Feed consumption (FC) was recorded on d 7, 14, and 20. Productivity index (PI) was calculated by using the following formula:

$$\mathrm{PI} = (100 - \mathrm{Mortality}) \times (\frac{\mathrm{BW}}{1000})/\mathrm{Bird\ Age}/\mathrm{FCR} \times 100$$

Blood cholesterol, and intestinal ph and viscosity

Peripheral blood samples were collected at d 20. Blood samples were centrifuged to obtain blood serum and the serum samples were then analyzed to obtain the blood cholesterol level by the Texas A&M Veterinary Medical Diagnostic Laboratory (TVMDL). Two birds were randomly selected from each pen and euthanized for harvesting of the gizzard, duodenum, jejunum, and ileum at d 20. The pH of the four organs were measured with a pH meter (Schott Instruments, Lab 850, Germany). Digesta viscosity samples were evaluated as described by Lee et al. (2003). Briefly, jejunal and ileal digsta were centrifuged at 4,500 × g for 20 min, then supernatants were aliquoted and placed in a viscometer (Brookfield Cone and Plate Viscometer4 with a CPE-40 spindle), then spindled at 37.8 °C for 20 seconds at 5 rpm.

Table 6.1. Experimental diets and nutrient composition

	1-21 d			
	CON[4]	ENZ[5]	GUAR[6]	BOTH[7]
Ingredients, %				
Corn, yellow grain	43.40	43.40	41.55	41.55
Soybean meal, dehulled solvent	39.46	39.46	30.64	30.64
Wheat Midds	6.00	6.00	6.00	6.00
DL Methionine	0.36	0.36	0.37	0.37
L-lysine	0.07	0.07	0.09	0.09
Fat, Blended A/V	5.80	5.80	6.84	6.84
Limestone	2.66	2.66	2.53	2.53
Bio-Phos 16/21 P	1.24	1.24	1.25	1.25
Salt	0.42	0.42	0.43	0.43
Trace Mineral[1]	0.05	0.05	0.05	0.05
Vitamins[2]	0.25	0.25	0.25	0.25
Guar Hull Fraction[3]	-	-	10.00	10.00
Nutrient Composition				
Crude Protein, %	24.00	24.00	24.00	24.00
ME, kcal/kg	3038	3038	3038	3038
Crude Fat, %	7.99	7.99	8.59	8.59
Lysine, %	1.38	1.38	1.33	1.33
Methionine, %	0.70	0.70	0.70	0.70
Cysteine, %	0.38	0.38	0.38	0.38
Tryptophan, %	0.30	0.30	0.29	0.29
Threonine, %	0.90	0.90	0.87	0.87
Arginine, %	1.61	1.61	1.76	1.76
Valine, %	1.09	1.09	1.04	1.04
Calcium, %	1.33	1.33	1.33	1.33
Phosphorus, %	0.68	0.68	0.70	0.70
Sodium, %	0.19	0.19	0.19	0.19
Analyzed Composition				
Crude Protein, %	22.95	23.15	22.96	23.55

[1] Trace mineral premix added at this rate yields 149.6 mg manganese, 55.0 mg zinc, 26.4 mg iron, 4.4 mg copper, 1.05 mg iodine, 0.25 mg selenium, a minimum of 6.27 mg calcium, and a maximum of 8.69 mg calcium per kg of diet. The carrier is calcium carbonate, and the premix contains less than 1% mineral oil.

[2] Vitamin premix is added at this rate yields 11,023 IU vitamin A, 3,858 IU vitamin D_3, 46 IU vitamin E, 0.0165 mg B_{12}, 5.845 mg riboflavin, 45.93 mg niacin, 20.21 mg d-pantothenic acid, 477.67 mg choline, 1.47 mg menadione, 1.75 mg folic acid, 7.17 mg peroxidase, 2.94 mg thiamine, 0.55 mg biotin per kg diet. The carrier is ground rice hulls.

[3]The nutrient matrix used was: crude protein, 35.4%; metabolizable energy, 2,100 kcal/kg; methionine, 0.44%; lysine, 1.54%; calcium, 0.16%; and available phosphorus, 0.16% (Lee et al., 2003).

[4]Control treated group.

[5]Control + 0.10 % of β-mannanase treated group.

[6]Contorl + 10 % of Guar hull fractions treated group.

[7]Control + 10 % of Guar hull fractions + 0.10 % of β-mannanase treated group.

[5]Control + 10 % of Guar hull fractions + 0.10 % of β-mannanase treated group.

Digesta passage rate

To measure digesta passage rate, the procedure used was adopted from Svihus et al. (2002). On d 21, immediately after the lights were turned on, all treatment feed trays were taken out replaced with feed that contained 5 g/kg of Titanium (IV) Oxide (TiO_2) as a marker. Birds were allowed to consume feed containing TiO_2 for 15 m, thereafter the original feed trays were returned to the pens. After the birds were allowed to consume TiO_2 containing feed for 30, 60, 90, and 120 min, two birds per pen from two replicates (4 birds per treatment) were randomly selected at each time point and humanely euthanized by CO_2 asphyxiation. Four gastrointestinal (gizzard, duodenum, jejunum, and ileum) digesta samples were collected. Digesta were stored at -20 °C until analyzed for TiO_2. Concentrations of TiO_2 in ashed samples were analyzed using the method described by Svihus et al. (2002). Briefly, the digesta contents of digestive tract segments were gently squeezed by hand, samples were then dried (Dryer, Sheldon manufacturing, Cornelius, OR) 24 hrs at 105 °C. The dried samples were ashed (Vulcan 3-1750 NEY Muffle furnace, Thomas Scientific, Swedesboro, NJ) 13 hrs at 550 °C. A calibration curve was established as described by Short et al. (1996). Briefly, 0.5 mg/ml of TiO2 concentration was added into 0, 1, 2, 3, 4, 5, 6, 7, 8, 9, 10 ml of distilled water to make standard titanium dioxide solution. TiO_2 concentration was measured by spectrometer (Genesys 10S UV-Vis, Thermo Fisher Scientific Inc., Madison, WI) at 210 nm. The concentration of TiO_2 was analyzed and the slope value between each digestive tract section was calculated using Microsoft Excel 2016 (Microsoft Corporation, Redmond, WA).

Statistical analysis

Data were analyzed via One-Way Analysis of Variance (ANOVA) for Completely Randomized Block Design (CRBD) using the Standard Least Squares procedure of JMP (JMP Pro® 12.0.1 for Windows, SAS Institute Inc., Cary, NC). Means were deemed significantly different at $P \leq 0.05$ and separated using the Least Squares Mean Differences Student's t-test.

Results and discussion

Growth performances

Mortality, average body weight (g), weight gain (g), cumulative and phase of the feed conversion ratio, and productivity index were observed to identify the effects of β-mannanase on ducklings. There was no mortality during this experiment.

Table 6.2 presents the results of body weight (BW), body weight gain (BG), and feed consumption (FC). At d 7, a significant difference ($P < 0.0001$) in BW was observed between ENZ and all other groups. The BW of the BOTH treatment was significantly less than CON. At d 14, ENZ had significantly greater BW ($P < 0.0001$) than all other treatments. GUAR had significantly lower BW than CON or BOTH at d 14. There was no significant difference in BW between CON and BOTH. The ENZ treatment continued to show a significant difference (P =0.0009) for BW at 21d compared to all other treatments. The pattern of significant differences among the treatments was identical to that of d 14. The pattern of significant differences in WG at d 7 among the treatments was identical to that described for BW at d7. At d 14, ENZ had significantly greater ($P = 0.0007$) WG than GUAR and BOTH. There was no significant difference between CON and BOTH. There

were no significant differences among the treatments (P = 0.1252) on d 21. CON and ENZ consumed significantly ($P < 0.0001$) more feed than GUAR and BOTH at d 7 and BOTH consumed significantly more feed than GUAR. At d 14, ENZ consumed significantly ($P = 0.0004$) more feed than GUAR and BOTH, but there was no significant difference between CON and BOTH. BOTH consumed significantly more feed than GUAR. There were no significant (P = 1018)

Table 6.2. Effect of β-mannanase on body weights, weight gain, feed consumption per bird from d 1-21 in Pekin ducks

Treatment	Body weight (g)				Weight gain (g)			Feed Consumption (g)		
	d1	d7	d14	d21	d7	d14	d21	d7	d14	d21
CON[1]	61.25	216.29[b]	714.5[b]	1302.34[b]	155.04[b]	498.21[ab]	587.83	294.21[a]	563.63[ab]	697.21
ENZ[2]	61.08	244.17[a]	781.04[a]	1412.38[a]	183.08[a]	536.88[a]	631.34	297.71[a]	601.86[a]	710.29
GUAR[3]	61.08	182.17[c]	595.5[c]	1137.67[c]	121.08[c]	413.33[c]	542.17	263.75[c]	473.63[c]	613.46
BOTH[4]	61.33	193.54[c]	680.46[b]	1266.38[b]	132.21[c]	486.92[b]	585.92	275.96[b]	539.04[b]	667.42
Pooled SEM		5.05	16.81	32.98	5.02	14.47	23.6	3.08	14.12	26.49
Treatment	N/A	0.0001	0.0001	0.0009	0.0001	0.0007	0.1252	0.0001	0.0004	0.1018

[a–c] Different letters in the same column indicate a significant difference ($P \leq 0.05$).
[1]Control treated group.
[2]Control + 0.10 % of β-mannanase treated group.
[3]Contorl + 10 % of Guar hull fractions treated group.
[4]Control + 10 % of Guar hull fractions + 0.10 % of β-mannanase treated group.

differences among the treatment in d 21 feed consumption. From d 14 to the end of the experiment, BW, WG, and FC of CON and BOTH treatments were not significantly different. Table 6.3 presents the results of phase (pFCR) and cumulative (cFCR) feed conversion ratio, and productivity index (PI). ENZ had significantly ($P = 0.0016$) lower d 0-7 pFCR than all other groups and there was no difference between CON and BOTH or between GUAR and BOTH. No significant differences existed among the treatments for d 7-14 (P = 0.1894) or d 14-21 (P = 0.8241) pFCR. BOTH had significantly ($P = 0.0006$) lower d 0-14 cFCR than all other groups. There was no significant difference between CON and BOTH, but d 0-14 cFCR of GUAR was significantly greater than all other groups. ENZ had a significantly greater productivity index than all other treatments at d 7 ($P < 0.0001$), d 14 ($P < 0.0001$), and d 20 ($P = 0.0038$). PI of CON at d 7 was significantly greater than GUAR and BOTH. There were no significant differences in PI between CON and BOTH at d 14 and d 20. The only significant difference in pFCR, cFCR, and PI between CON and BOTH was d 7 PI. Indicating that β-mannanase supplementation of guar containing duck diets can ameliorate the negative effects of high levels of NSPs. Comparable results were observed in another study (Lee et al., 2003). The researchers also started to observe the effects of β-mannanase on BW and cFCR in broiler chickens clearly at d 14. They used 0, 2.5, and 5 % of guar hull fraction with three different levels (none, low, and high) of β-mannanase in broiler chicken diets. The results of the present study in regard to BW and cFCR are consistent with those reported by Lee et al. (2003).

Table 6.3. Effect of β-mannanase on feed conversion ratio and productivity index from d 7-21 in Pekin ducks

Treatment	Phase FCR			Cumulative FCR		Productivity Index		
	d0 to 7	d7 to 14	d14 to 21	d0 to 14	d0 to 21	d7	d14	d21
CON[1]	1.90[b]	1.13	1.18	1.31[b]	1.25	163[b]	388[b]	519[b]
ENZ[2]	1.63[c]	1.12	1.14	1.25[c]	1.20	214[a]	446[a]	594[a]
GUAR[3]	2.18[a]	1.15	1.13	1.38[a]	1.25	119[c]	308[c]	453[c]
BOTH[4]	2.11[ab]	1.11	1.14	1.32[b]	1.23	133[c]	369[b]	515[bc]
Pooled SEM	0.08	0.01	0.05	0.015	0.02	8.52	12.84	20.06
Treatment	0.0016	0.1894	0.8241	0.0006	0.3097	0.0001	0.0001	0.0038

[a–c] Different letters within the same column indicate a significant difference ($P \leq 0.05$).
[1]Control treated group.
[2]Control + 0.10 % of β-mannanase treated group.
[3]Contorl + 10 % of Guar hull fractions treated group.
[4]Control + 10 % of Guar hull fractions + 0.10 % of β-mannanase treated group.

The effects of addition of β-mannanase in duck diets containing guar was examined in this study. β-mannanase started to show its effect in guar treated feed at d 14. Guar is known to disturb host digestive tract development. It is apparent that 0.1 % of β-mannanase is not enough to overcome problems associated with guar in the digestive tracts of early age ducks. However, these results confirm that β-mannanase can replace normal corn-soybean meal feed for growth performance in White Pekin duck from d 14 -21.

Gastrointestinal ph, cholesterol level in blood, and intestinal viscosity

GUAR had significantly ($P = 0.0006$) higher jejunal viscosity than all other groups (Table 6.4). CON and ENZ had significantly ($P < 0.0001$) lower ileal viscosity than BOTH which in turn was significantly lower than GUAR. There was no significant difference in jejunum viscosity between CON and BOTH, indicating that β-mannanase supplementation restores jejunal digesta viscosity to normal levels. Similar results were reported by Lee et al. (2003). The authors observed that addition of β-mannanase to broiler diets containing 5% guar hull fractions resulted in significantly lower ileal viscosity in the enzyme treated groups. Gizzard, jejunum, and ileum pH were influenced by β-mannanase supplement (Table 4). ENZ had significantly ($P = 0.0204$) higher gizzard pH than GUAR. No other treatment comparisons were significantly different. There was no significant difference in the pH of the duodenum among the groups. ENZ and BOTH had significantly higher jejunum ($P = 0.0063$) and ileum ($P = 0.0012$) pH values than CON and GUAR. These results indicate that guar hull fraction or non-starch polysaccharides reduced pH of the digestive tract. These results show similar patterns

Table 6.4. Effect of β-mannanase on gastrointestinal viscosity (cP), pH, and cholesterol level (mg/dL) of Pekin ducks

Treatment	Viscosity		pH				Cholesterol level (mg/dL)
	Jejunum	Ileum	Gizzard	Duodenum	Jejunum	Ileum	
CON[1]	3.64b	4.05c	3.95ab	5.57	5.76b	6.17b	133.33
ENZ[2]	3.10b	2.99c	4.48a	6.07	6.86a	7.67a	142.33
GUAR[3]	11.84a	33.37a	3.39b	5.36	5.63b	6.45b	107.67
BOTH[4]	4.60b	11.96b	3.96ab	5.88	6.57a	7.27a	122.67
Pooled SEM	1.12	2.49	0.20	0.20	0.22	0.20	7.7
Treatment	0.0006	0.0001	0.0204	0.1348	0.0063	0.0012	0.1488
Room	0.9783	0.4993	0.1097	0.9325	0.0346	0.2910	0.8369

[a-c] Different letters within the same column indicate a significant difference ($P \leq 0.05$).
[1]Control treated group.
[2]Control + 0.10 % of β-mannanase treated group.
[3]Contorl + 10 % of Guar hull fractions treated group.
[4]Control + 10 % of Guar hull fractions + 0.10 % of β-mannanase treated group.

within intestinal viscosity and pH. In a high gut viscosity environment, anaerobic bacteria populations will be increased and will release more acetic acid than a healthy gut environment (Choct, 1997). Acetic acid has the effect of lowering the pH of the digestive tract. However, if β-mannanase is present in the duck feed, digestive tract pH will be increased because β-mannanase breaks down the non-starch polysaccharides backbone. There is also the possibility that bile salt may be bound or trapped in the duodenum by high gut viscosity (Moundras et al., 1997). In the duodenum, there are few changes in microflora because it is where digestive enzymes and antimicrobial (such as bile salts) activities occur most frequently along the digestive tract (Gabriel et al., 2006). For this reason, the duodenum seems to have no significant pH change according to our results, and β- mannanase treated groups had significantly higher pH in the jejunum and the ileum than non-β- mannanase treated groups possibly due to binding of bile salts in the duodenum. Unlike our pH result, Houshmand et al. (2011) and Hernandez et al. (2006) did not observe any differences in pH of the digestive tract. These authors used pre-biotics, pro-biotics and organic acids in broiler chicken based studies, respectively. Their results showed that other supplementations did not impact digestive pH level, but our data demonstrates that enzymes can impact digestive tract pH. Enzymes have influence on the digestive tract pH level because of the hydrolysis effect that breaks down the backbone of the non-polysaccharides. The reason why the results are different could also be caused by species differences. Mabelebele et al. (2014) analyzed pH of the digestive organs of Ross 308 broilers and Venda chickens and they observed significant differences in pH

depending on the breeds; Venda chickens had significantly lower pH in the crop, gizzard, and small intestines.

There were no significant differences among the treatments in blood serum cholesterol level (Table 6.4). The results indicate that 0.1 % of β-mannanase may not be enough to increase cholesterol level in blood serum or 10 % of guar hull fraction may not be effective in reducing bioavailability of dietary minerals and fat with binding bile salt in the digestive tract of ducks. In contrast to our results, Zarghi and Golian (2009) observed that multi enzyme (xylanase and β-glucanase) increased blood serum cholesterol in d 42 broiler chickens. Frigard et al. (1994) also observed differences in blood serum cholesterol levels (HDL/Total serum cholesterol) between the groups using the enzyme and the non-enzyme groups by the age of the chickens. These authors observed that blood serum cholesterol level differences were affected by the enzyme in d 21 broiler chickens, but cholesterol levels were not different in d 15 chickens. In our study, duckling blood serum samples were collected at d 20. It is possible that d 20 ducklings were too young to elicit a response, thus no significant difference was observed in serum cholesterol levels among the groups in our experiment.

Feed passage rate

There was no significant difference in feed consumption during the 120 min exposure to feed containing TiO2 (Data not shown). Table 6.5 presents the concentration of TiO_2 in each digestive tract section by time and Table 6.6 presents slope value comparison between digestive tract sections in time. Concentration of TiO_2 in the gizzard showed no significant difference among the various treatments in this study. No

Table 6.5. Titanium (IV) Oxide (TiO2) concentration (mg) in gastorointestinal digesta of White Pekin ducks as affected by time (min) after given access to each diet containing TiO2

Treatment	TiO2 in Gizzard				TiO2 in Duodenum				TiO2 in Jejunum				TiO2 in Ileum			
	30	60	90	120	30	60	90	120	30	60	90	120	30	60	90	120
CON[1]	19.3	12.7	4.2	16.5	1.2	1.9	2.4	0.6	9.7	10.5^{b}	11.0	9.4	5.7	11.5	34.1^{b}	35.2^{b}
ENZ[2]	20.3	8.9	5.4	14.8	1.2	2.0	0.8	0.8	6.4	4.5^{c}	6.8	5.4	3.6	8.0	47.1^{a}	48.0^{a}
GUAR[3]	12.7	14.4	9.5	8.0	2.4	3.3	0.8	1.2	9.0	16.4^{a}	4.1	4.3	2.8	10.6	27.1^{b}	33.4^{b}
BOTH[4]	17.0	23.5	7.5	8.4	0.8	2.5	1.9	1.1	3.9	6.0^{bc}	4.6	6.8	3.1	9.2	39.3^{ab}	46.2^{a}
Pooled SEM	2.44	5.89	3.05	5.54	0.76	0.52	0.37	0.21	2.42	1.27	2.29	4.17	0.45	2.19	2.85	1.25
Treatment	0.30	0.47	0.66	0.65	0.55	0.36	0.12	0.28	0.44	0.02	0.31	0.84	0.06	0.71	0.05	0.01

[a–c] Different letters within the same column indicate a significant difference ($P \leq 0.05$).
[1]Control treated group.
[2]Control + 0.10 % of β-mannanase treated group.
[3]Contorl + 10 % of Guar hull fractions treated group.
[4]Control + 10 % of Guar hull fractions + 0.10 % of β-mannanase treated group.

Table 6.6. Slope value (linear regression) that presents Titanium (IV) Oxide (TiO2) concentration (mg) in gastorointestinal digesta of White Pekin ducks as affected by time

Treatment	60 minutes				90 minutes			
	G to IL[5]	G to D[6]	D to J[7]	J to IL[8]	G to IL[5]	G to D[6]	D to J[7]	J to IL[8]
CON[1]	0.5106	-10.782	8.6037[b]	1.0125	9.8176[ab]	-1.8138	8.5797	23.0995[b]
ENZ[2]	-0.0163	-6.900	2.4856[c]	3.5317	13.1335[a]	-4.6161	6.0701	40.3020[a]
GUAR[3]	0.1790	-11.056	13.0470[a]	-5.7438	5.6061[ab]	-8.7138	3.2582	23.0565[b]
BOTH[4]	-3.9353	-20.984	3.5173[c]	3.1765	9.7944[b]	-5.6622	2.7064	34.7025[a]
Pooled SEM	1.59	5.46	0.87	1.84	1.00	2.88	2.01	2.00
Treatment	0.3323	0.4388	0.0092	0.0986	0.0487	0.5061	0.3172	0.0191

[a–c] Different letters within the same column indicate a significant difference ($P \leq 0.05$).
[1]Control treated group.
[2]Control + 0.10 % of β-mannanase treated group.
[3]Contorl + 10 % of Guar hull fractions treated group.
[4]Control + 10 % of Guar hull fractions + 0.10 % of β-mannanase treated group.
[5]Slope value between gizzard and ileum slope value
[6]Slope value between gizzard and duodenum slope value
[7]Slope value between duodenum and jejunum slope value
[8]Slope value between jrjunum and ileum slope value

significant differences were found among the treatments in TiO2 concentration in the duodenum. At 60 min, GUAR jejunum contained significantly greater (P = 0.02) TiO2 than other groups. The concentration slope value (Table 6.6) between the duodenum and the jejunum at 60 min showed that GUAR had a higher (P = 0.0092) slope value than all other groups, and CON had a higher slope value than ENZ and BOTH. In the ilium at 90 min the ENZ group had greater (P = 0.05) TiO2 concentration than CON and GUAR groups. These differences persisted at 120 min. ENZ and BOTH treatments had significantly greater ($P \leq 0.01$) TiO2 concentration in the ileum compared to CON and GUAR at 120 min. Concentration slope value between the gizzard and the ileum at 90 min showed that ENZ had significantly higher (P = 0.0487) slope value than GUAR (Table 6.6). The concentration slope value between the jejunum and the ileum at 90 min (Table 6.6) revealed that ENZ and BOTH had significantly higher (P = 0.0191) slope values than CON and GUAR. At 60 min, enzyme-treated groups contained less TiO_2 concentration in the jejunum than non-enzyme-treated groups. However, the TiO_2 concentration of ENZ and BOTH increased smoothly between jejunum and ileum samples, while TiO_2 concentration of CON did not increase much and GUAR stagnated (Figure 6.2). This result appears to be due to the high jejunal viscosity of the GUAR treatment group. High digesta viscosity in the jejunum may have caused a decreasing digesta passage rate. The 90 and 120 min ileum TiO_2 concentrations of CON and GUAR were lower than ENZ and BOTH due to the decreasing jejunem digesta passage rate of the CON and GUAR treatments at 60 min. Figures 6.1, 6.2, 6.3, and 6.4 present the quadratic coefficient value (CV) between gizzard and ileum at 30, 60, 90, and 120 min,

Figure 6.1. Quadratic coefficient value between gizzard to ileum at 30 minutes

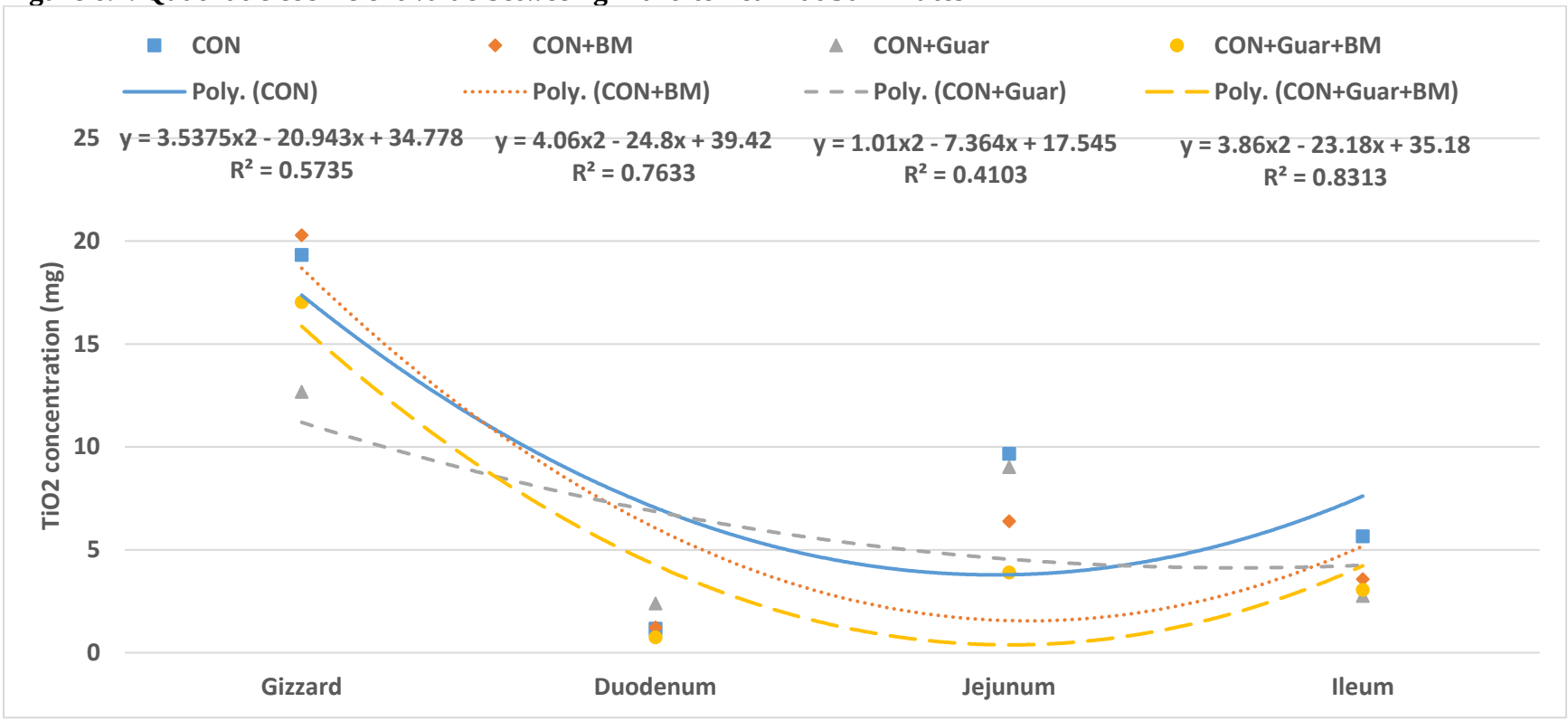


Figure 6.2. Quadratic coefficient value between gizzard to ileum at 60 minutes

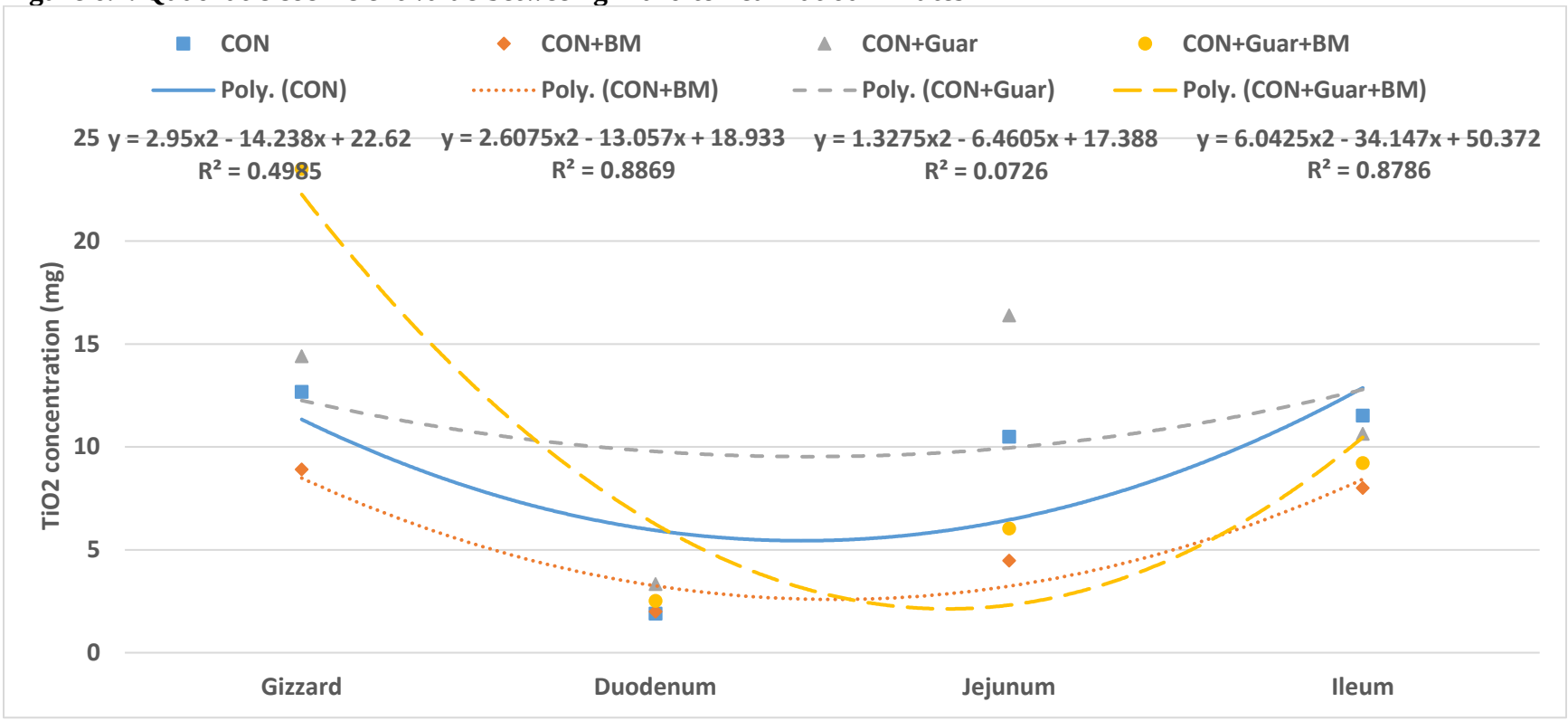


Figure 6.3. Quadratic coefficient value between gizzard to ileum at 90 minutes

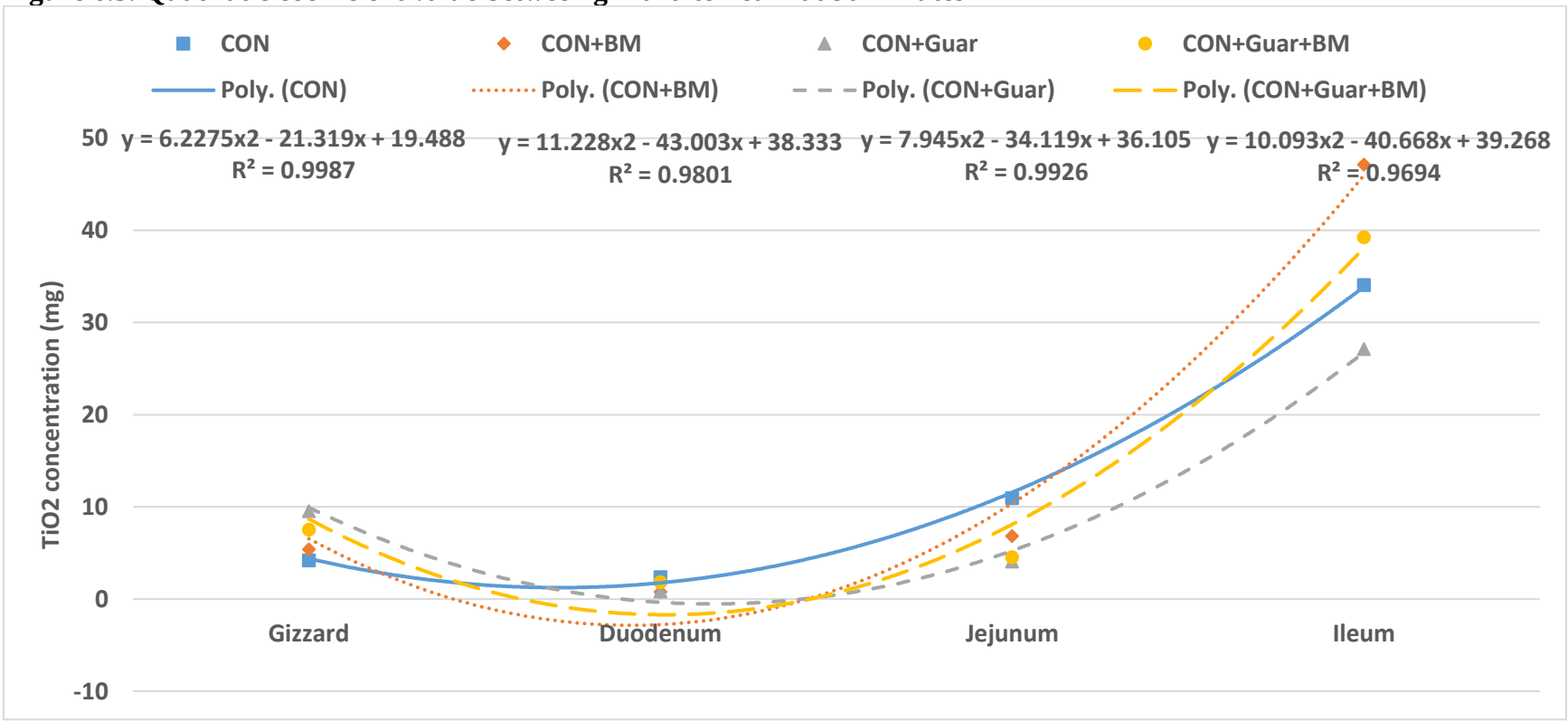
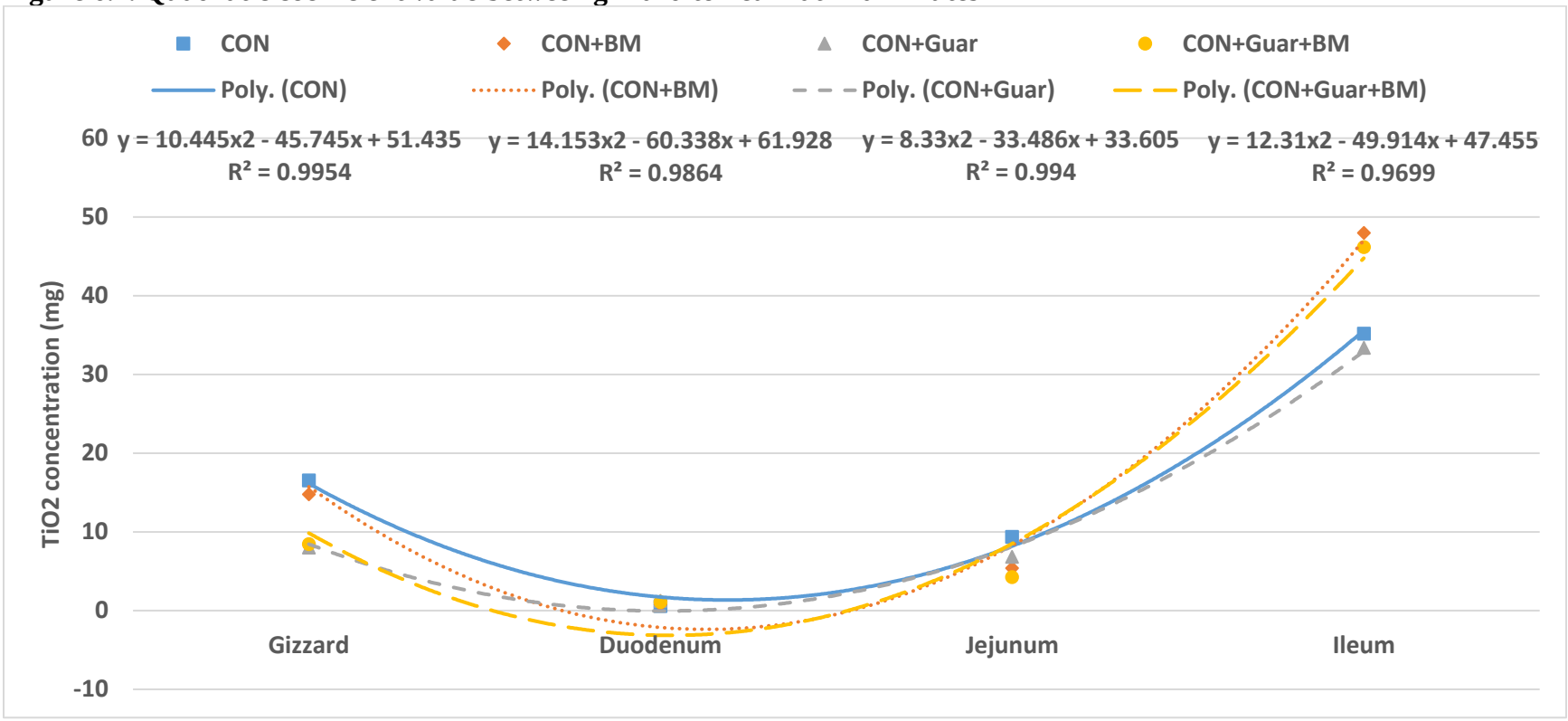


Figure 6.4. Quadratic coefficient value between gizzard to ileum at 120 minutes



respectively. GUAR always had a lower CV than all other groups at 30, 60, and 120 min. On the other hand, the ENZ treatment was observed to have a higher CV than all other groups at 30, 90, and 120 min. The BOTH treatment was also observed to have a higher CV than CON and GUAR at all time points. These results further indicate the impact of the enzyme treatment. BOTH had a better CV than the CON at all points.

Table 6.7 presents quadratic regression coefficient for TiO2 concentration (mg) from gizzard to ileum. No significant differences were observed among the groups at 30 or 60 min. ENZ had significantly (P = 0.0335) greater CV than CON and GUAR at 90 min, there was no significant difference between GUAR and BOTH, and between CON and GUAR. At 120 min ENZ had significantly (P = 0.0464) greater CV than CON and GUAR, there was no significant difference between CON and BOTH, and between CON and GUAR. At 90 and 120 min there were no significant differences between CON and BOTH, further indicating that the enzyme ameliorates the impact of NSPs on digesta passage rate.

Unlike most commercial poultry, ducks do not have crops. The reason that the TiO_2 concentration remained high in the gizzard is due to the fact that the first digestive organ of ducks is the gizzard. Other studies based on broiler chickens (Vergara et al., 1989; Barash et al., 1993) also observed similar trends. The gizzard is a muscular digestive organ and it only grinds until feed particles are smaller than a certain size (Svihus et al., 2002; Moore. 1999). Several experiments (Kiiskinen, 1996; Waldenstedt et al., 1998) reported that the size of this was correlated to feed intake. In our study, size or weight of gizzard was not measured, but there were no significant differences in TiO_2

Table 6.7. Coefficient value (quadratic regression) that presents Titanium (IV) Oxide (TiO2) concentration (mg) in gastorointestinal digesta (gizzard to ileum) of White Pekin ducks as affected by time

Treatment	30 minutes	60 minutes	90 minutes	120 minutes
CON[1]	3.5377	2.9487	6.2284[c]	10.4440[bc]
T10[2]	4.0595	2.6080	11.2295[a]	14.1520[a]
CONG[3]	1.0113	1.3280	7.9427[bc]	8.3301[c]
T10G[4]	3.8604	6.0401	10.0911[ab]	12.3070[ab]
SEM	0.5477	1.3314	0.6303	0.7973
Treatment	0.0759	0.2610	0.0335	0.0464
Room	0.3161	0.4214	0.0433	0.9477

[a–c] Different letters within the same column indicate a significant difference ($P \leq 0.05$).
[1]Control treated group.
[2]Control + 0.10 % of β-mannanase treated group.
[3]Contorl + 10 % of Guar hull fractions treated group.
[4]Control + 10 % of Guar hull fractions + 0.10 % of β-mannanase treated group.

concentration in gizzard digesta among the groups. The digesta passage rate between duodenum and jejunum at 60 min of CON and GUAR was faster than ENZ and BOTH because CON and GUAR had a shorter duodenum length than ENZ and BOTH (Data not shown). The duodenum is one of the main areas that generate enzymes for feed digestion and there were no significant differences in digesta viscosity in this organ among the groups. Therefore, even though the digesta passage rate between gizzard to duodenum of CON and GUAR was faster than ENZ and BOTH, it would not have had a significant effect on nutrient digestion and absorption. GUAR had a very high viscosity in jejunum and ileum, so GUAR had the lowest digesta passage rate which was expected.

Conclusion

This study was conducted to verify the effects of β-mannanase with and without high dietary non-starch polysaccharides. According to the results, β-mannanase impacted digesta passage rate, pH of digestive tracts, and live performance. The addition of β-mannanase supplementation in guar hull fraction treated group had statistically equivalent values when compared with basal corn-soybean meal treated groups in digesta passage rate (90 and 120 min), jejunal pH, and live performance after d 14. In conclusion, addition of a supplement can replace the normal corn-soybean meal feed, even if β-mannanase is used in feed that contains a high concentration of non-starch polysaccharides.

CHAPTER VII

SUMMARY AND CONCLUSION

Antibiotics have been helpful for improving growth performance of poultry and increasing resistance to certain diseases. However, the use of antibiotics has been banned because if poultry feed contains antibiotics there is a chance that residues in poultry meat could be transferred to humans when they consume poultry meat. Then it could alter the immune system of humans. Since antibiotics were banned in the poultry industry, efforts to find feed additive that alternate the antibiotics has been increased. Now it is important to increase efficiency for increased revenue to produce more than before. Enzymes and yeast cell wall derived mannan-oligosaccharides (YCW-MOS) are popular feed additives that may substitute for antibiotics.

β-mannanase is one of the enzymes and is well-known to break back bones of non-polysaccharide (NSP) chains. After β-mannanase breaks down NSPs back bone, the mannose or YCW-MOS are released as residue. Therefore, one of my hypothesis was that YCW-MOS as a feed additive may need to be added more into the poultry feed than β-mannanase. According to my results, I found that using β-mannanase has more merit than using YCW-MOS for efficiency of price and meat produced because the required level of YCW-MOS for enhancing duck growth performance was higher than the level of β-mannanase. Even low levels of β-mannanase showed greater effects than YCW-MOS. For example, YCW-MOS showed improvement of growth performance, feed consumption, feed conversion, and productivity index (PI) at d 21 only. However, T250 and T2000 was not different than the CON in terms of duck feed consumption. All YCW-MOS-treated

groups, except T1000, were not different than the CON in terms of cumulative and phase of FCR. Ducks fed T250 and T500 did not have different PI compared to ducks fed the CON. Therefore, these data concluded that 1 kg/ton of YCW-MOS only impacted duck growth performance. On the other hand, addition of four different levels of β-mannanase in the diet of ducks positively impacted every single point of duck growth performance. As a result, β-mannanase supplementation appears to have more powerful effects than YCW-MOS.

Histomorphological results were similar to growth performance results. Addition of YCW-MOS to the diet impacted ileum villi height and crypt depth, jejunum goblet cell area, and jejunum and ileum goblet cell population. In this case, T1000 only showed differences with the CON in ileum crypt depth, jejunum goblet cell area, and ileum goblet cell number. Ducks fed diets T250, T500, and T2000 showed no differences to the CON in most of the histomorphological results. In the β-mannanase trial, ducks fed T10 had greater ileum villi height compared to those fed CON, T01, and T20. Ducks fed T05, T10, and T20 had greater ileum villi width compared to those fed CON. Also, ducks fed T05, T10, and T20 had greater ileum crypt depth compared to CON and T01; whereas, those fed T05, T10, and T20 had greater ileum goblet cell population compared to CON.

Cysteine, histamine, and tryptophan absorption and digestibility was improved by YCW-MOS. At results, 1 kg/ton of MOS could be the ideal level for the ducklings to derive better nutrient absorption and amino acid digestibility, among 250 g, 500 g, 1 kg, and 2 kg /ton of YCW-MOS. On the other hands, all four different levels of β-mannanase improved ileum amino acid digestibility compared to CON. Also, β-mannanase improved

fat percentage in body and bone strength of Pekin ducks. This study suggests that the 0.1 % of β-mannanase is the ideal level for ducklings to derive better nutrient absorption and amino acid digestibility.

β-mannanase not only improved growth performance, nutrient digestibility, gut morphologies, but also improved digesta passage rate, gut pH, and gut viscosity. To evaluate effects of β-mannanase in the digesta passage rate, gut pH, and viscosity, guar hull was used as a challenge because guar contains much more NSPs than normal corn-soybean meal diets. When β-mannanase was used with guar, the digesta passage rate, gut pH, and gut viscosity were improved significantly. Also, there were no significant differences in growth performance after d 14, gizzard pH, and jejunal viscosity between control and β-mannanase with guar treated group. Although guar contains more NSPs than corn-soybean meal, the addition of β-mannanase in the guar hull showed no significant differences with the control group.

The effects of β-mannanase were revealed from various responses of ducks in the various experiments. β-mannanase seems to have a lot of potential positive effects on poultry nutrition, but this study did not test other aspects, such as immune system, gene expression, or metabolic signaling system. More experiments are needed to address these responses.

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