DIETARY FIBER IN THE NUTRITION OF THE BROILER CHICKEN

by

OSCAR JONATHAN TEJEDA MARTINEZ

(Under the Direction of Woo Kyun Kim)

ABSTRACT

Carbohydrates are the largest component of a typical poultry diet and, potentially, the least understood nutrients, especially dietary fiber (DF). Therefore, a series of 4 experiments was conducted to determine the main role of DF in the entire nutrient metabolism in broiler chickens. The first experiment (chapter 3) utilized increasing levels of cellulose and soyhulls as sources of dietary fiber to achieve 4, 6, and 8% crude fiber (CF). Soyhulls had a more substantial impact improving intestinal histomorphology and performance parameters compared to cellulose when added at 4% CF. The second experiment (chapter 4) attempted to replicate different soluble (SF) to insoluble fiber (IF) ratios using semi-purified diets. It was clearly observed that higher soluble to insoluble fiber ratios interfere with normal nutrient digestibility and increase intestinal viscosity and that replacement of SF with IF recovers performance parameters and nutrient digestibility. To better understand the role of physical structure of dietary fiber, the third experiment (chapter 5) investigated the use of diets varying in fiber type, particle size, and inclusion level, yet maintaining the same nutrient levels. Among fiber-containing treatments, weight gain was better for soyhull-containing diets with a fine particle and lower inclusion level (i.e. 4%). Coarse particles of soyhulls increased gizzard relative weight. The fourth experiment (chapter 6) utilized pelleted diets and two sources of soybean meal, namely, the typical 48%

crude protein soybean meal and the hulled 44% crude protein soybean meal to which soyhulls were added. Results from this experiment indicate that inclusion of hulled 44% soybean meal can positively modulate intestinal histomorphology parameters and expression of immune genes without compromising performance. Altogether, these results indicated that dietary fiber is an important dietary component modulating nutrient metabolism and general physiology in broiler chickens. Based on the results from these experiments it is concluded that soybean hulls can be added to diets of broiler chickens at a maximum level of 6%. Future research should strive to stablish the adequate inclusion level of other fibrous ingredients in order to develop practical and cheaper diets aimed at reducing the production costs in the broiler industry.

INDEX WORDS: dietary fiber; broiler chicken; intestinal morphology; nutrient digestibility; intestinal viscosity; nutrient transporters; particle size; 44% soybean meal.

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DEDICATION

To THE KING OF KINGS and LORD of Lords, JESUS CHRIST my LORD, GOD, and SAVIOUR, for all things were made by HIM; and without HIM was not anything made that was made. For THE LORD giveth wisdom: out of HIS mouth cometh knowledge and understanding. HE is the One Who opens doors that no man can shut and the One Who has led me to be able to achieve all these goals. For of HIM, and through HIM, and to HIM are all things; to WHOM be the glory forever. There are not enough words to thank THE LORD JESUS for all that HE has done, nor enough praises to demonstrate my gratitude for HIS faithfulness. I can surely say, hitherto hath JEHOVAH helped me.

After GOD, I am the person I am now, thanks to my parents, Luis and Rosa Tejeda, my brother Luis and my sister Yeslin Tejeda. Finally, this is also dedicated to my grandmother on heavens, as I promised I would, and this is just the beginning.

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My heartfelt thanks are for all my family. It would be misleading to let this dissertation have my name alone in it, because I am not solely responsible for it. I thank my family for the innumerable sacrifices, constant support, and prayers that have been a motivation to keep moving forward. With this, I can only thank THE LORD JESUS for the time has come.

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CHAPTER 1

INTRODUCTION

The demand for chicken meat has increased substantially in the last seven decades due to structural and organizational changes in the poultry industry (National Chicken Council, 2013). Much of this increase in production is attributed to improvements in genetic selection and nutrition (Havenstein et al., 1994). With these increases in production there is an evident need for the seeking of alternative feed ingredients that keep or improve the production efficiency and that are constantly available to be utilized as cheap nutrient sources (Mumm et al., 2014). However, one of the challenges faced when using alternative feed ingredients is their high content of fibers (Jaworski et al., 2015) whose use is generally evaded by nutritionists due to misconceptions and lack of understanding of their physiological role which has led to rejection of fibrous materials.

The recent improvements in feed formulation have allowed the poultry industry to maximize the utilization of nutrients from feedstuffs and at the same time reduce the environmental impact (Leinonen and Kyriazakis, 2016). The new formulation systems utilized for broilers are based on digestible nutrients where every single metabolite is scrutinized before being set as part of the nutrient matrix (Choct, 2015). Interestingly, the fact that carbohydrates make the largest portion of any poultry specie, does not necessarily mean that carbohydrates are understood completely, especially dietary fiber. Dietary fiber is a component of vegetable feedstuffs that is found in different types and amounts (Knudsen, 2014). Dietary fibers are composed of indigestible carbohydrates including polysaccharides (i.e. cellulose, hemicellulose),

oligosaccharides (i.e. stachyose, raffinose), and others non-carbohydrates components (i.e. lignin) with the ability to resist digestion and absorption in the gastrointestinal tract (Prosky, 2000). This ability to pass through the gastrointestinal tract without being absorbed, gives the dietary fibers the opportunity to interact with the bolus in the gastrointestinal tract and, depending on the particle size, modulate digestive organ growth that can result in changes in nutrient digestibility (González-Alvarado et al., 2007; Sacranie et al., 2012). Several reports have indicated that DF can improve the digestibility of starch (Hetland and Svihus, 2001), amino acids (Kluth and Rodehutscord, 2009; Tejeda and Kim, 2020), and the retention of dry matter, nitrogen, ether extract and AME_n (Jiménez-Moreno et al., 2009). There are several reports indicating that dietary fibers can improve growth performance when included at 3-5% in broiler diets as a result of changes in nutrient metabolism (Hetland and Svihus, 2001; Rezaei et al. 2018; Sklan et al., 2003). Furthermore, dietary fibers cannot be digested by broilers, but it can be degraded by intestinal microbes which results in changes in intestinal microbiota depending on the type of carbohydrate being fermented (Józefiak et al., 2010). This is of crucial importance since the removal of antibiotics from poultry diets has led the poultry industry to attempt the shift of microbial populations toward healthier bacterial populations in an effort to maintain performance efficiency (Choct, 2009).

The biggest challenges about dietary fibers in poultry diets is understanding the physiological and nutritional role exerted by fibrous components based on the type and amount of fiber and with this, finding the adequate fiber levels that positively modulate intestinal health and development (Choct, 2015; Tejeda and Kim, 2021). The lack of consistency in the results presented by other researchers when using higher-than-normal inclusions of DF in broiler diets could be attributed to the variability in nutrient content of the experimental diets, the amount

used, or even the type of fiber used (Tejeda and Kim, 2021). Therefore, additional research is warranted to further understand the role of dietary fiber in the nutrition of the broiler chicken.

The work presented herein consists of 7 chapters and aims to elucidate the role of dietary fiber on the nutrition of the broiler chicken from a nutritional-physiological point of view. Chapter 2 is presented as a literature review and it links together already-published data regarding dietary fiber in different poultry species. Furthermore, chapter 2 exposes data that are important to continue the understanding of the role of dietary fiber in poultry nutrition. The study of dietary fibers on the nutrition of the broiler chicken is presented as experiments in chapters 3, 4, 5, and 6. Data generated by these experiments should provide a basis for practical nutritional recommendations to maximize the functionality of dietary fiber when used in poultry diets. Therefore, chapter 7 links together all the data gathered during previous chapters, and provides recommendations to scientists/nutritionists working with fibrous ingredients in poultry, and to the industry on how to obtain the best results when using fibrous ingredients.

REFERENCES

Choct, M. 2009. Managing Gut Health through Nutrition. Br. Poult. Sci. 50: 9–15.

Choct, M. 2015. Fibre - Chemistry and Functions in Poultry Nutrition. LII Simp. Cient. Avi.: 113–119.

González-Alvarado, J. M., E. Jiménez-Moreno, R. Lázaro, and G. G. Mateos. 2007. Effect of type of cereal, heat processing of the cereal, and inclusion of fiber in the diet on productive performance and digestive traits of broilers. Poult. Sci. 86:1705–15.

Havenstein, G. B., P. R. Ferket, S. E. Scheideler, and D. V. Rives. 1994. Carcass Composition and yield of 1991 vs 1957 broilers when fed ‘typical’ 1957 and 1991 broiler diets. Poult. Sci. 73:1795–1804.

Hetland, H, and B Svihus. 2001. Effect of oat Hulls on performance, gut capacity and feed passage time in broiler chickens. Brit. Poult. Sci. 42:633–37.

Jaworski, N. W., H. N. Lærke, K. E. Bach Knudsen, and H. H. Stein. 2015. Carbohydrate composition and in vitro digestibility of dry matter and nonstarch polysaccharides in corn, sorghum, and wheat and coproducts from these grains. J. Anim. Sci. 93:1103–13.

Jiménez-Moreno, E., J. M. Ginzalez-Alvarado, A. Gonzalez-Serrano, R. Lazaro, and G. Mateos.. 2009. Effect of dietary fiber and fat on performance and digestive traits of broilers from one to twenty-one days of age. Poult. Sci. 88:2562–74.

Józefiak, D., A. Tutkowski, S. Kaczmarek, B. B. Jensen, R. M. Engberg, and O. Hojberg. 2010. Effect of β-glucanase and xylanase supplementation of barley- and rye-based diets on caecal microbiota of broiler chickens. Br. Poult. Sci. 51:546–57.

Kluth, H., and M. Rodehutscord. 2009. Effect of inclusion of cellulose in the diet on the inevitable endogenous amino acid losses in the ileum of broiler chicken. Poult. Sci. 88: 1199–1205.

Knudsen, K. E. B. 2014. Fiber and nonstarch polysaccharide content and variation in common crops used in broiler diets. Poult. Sci. 93: 2380–93.

Leinonen, I., and Ilias K. 2016. How can we improve the Eevironmental sustainability of poultry production? Proc. Nutr. Soc. 75:265–73.

Mumm, Rita H., Peter D. Goldsmith, Kent D. Rausch, and Hans H. Stein. 2014. Land usage attributed to corn ethanol production in the United States: sensitivity to technological advances in corn grain yield, ethanol conversion, and co-product utilization." Biotechnol. Biof. 7: –17.

National Chicken Council. 2021. Statistics and research: Per capita consumption of poultry and livestock, 1965 to estimated 2021, in pounds. Accessed January 2021. http://www.nationalchickencouncil.org/about-the-industry/statistics/per-capita-consumption-of-poultry-and-livestock-1965-to-estimated-2012-in-pounds/

Prosky, L. 2000. When Is Dietary Fiber Considered a Functional Food? BioFactors 12: 289–97.

Rezaei, M., M. A. Karimi Torshizi, H. Wall, and E. Ivarsson. 2018. Body growth, intestinal morphology and microflora of quail on diets supplemented with micronised wheat fibre." British Poultry Science 59(4): 422–29. https://doi.org/10.1080/00071668.2018.1460461.

Sacranie, A., B. Svihus, V. Denstadli, B. Moen, P. A. Iji, and M. Choct 2012. The effect of insoluble fiber and intermittent feeding on gizzard development, gut motility, and performance of broiler chickens. Poult. Sci. 91:693–700.

Sklan, D., A. Smirnov, and I. Plavnik. 2003. The effect of dietary fibre on the small intestines and apparent digestion in the turkey. Br. Poult. Sci. 44:735–40.

Tejeda, O. J., and W. K. Kim. 2020. The effects of cellulose and soybean hulls as sources of dietary fiber on the growth performance, organ growth, gut histomorphology, and nutrient digestibility of broiler chickens. Poult. Sci. 99:6828–36.

Tejeda, O. J., and Woo K. Kim. 2021. Role of dietary fiber in poultry nutrition. Anim. 11: 1–16.

CHAPTER 2

LITERATURE REVIEW: ROLE OF DIETARY FIBER IN POULTRY NUTRITION[1]

[1] Tejeda, O. J. and Kim, W. K. 2021. Role of dietary fiber in poultry nutrition. Anim. 11:1-16
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1. Carbohydrates and dietary fiber

Carbohydrates represent the biggest constituent of poultry diets and are one of the least understood feed components in broiler diets, especially dietary fiber (DF). Different reports regard DF either as a functional component for normal digestive organ functioning (Cao et al., 2003; Hetland et al., 2004; Jiménez-Moreno et al., 2009) or as an antinutrient (Gonzalez-Alvarado et al., 2008; Mateos and Serrano, 2012; Sadeghi et al.,). This is likely due to the complexity and variability of DF physical and chemical structure, which makes this portion of the diet more challenging to understand chemically and physiologically (Choct, 2015). From a nutritional perspective, DF has been defined as the group of heterogenous compounds, including all the saccharides (excluding starch, i.e. oligosaccharides, polysaccharides, pectins, gums, waxes) and lignin that resistant to enzymatic hydrolysis (Prosky, 2000; Trowell, 1972). A simpler definition of fiber describes DF as the sum of soluble and insoluble non-starch polysaccharides (NSP) and lignin (Choct, 2015). Notwithstanding of their composition, soluble fibers are avoided when formulating broiler diets since these are the type of fibers that increase intestinal viscosity, reducing the passage rate of the digesta through the gastrointestinal tract which can create hypoxic conditions in the intestinal tract that favors pathogenic bacteria growth (Langhout, 1998; Owusu-Asiedu et al., 2006). On the other hand, insoluble fibers, has been used as feed diluents in monogastric diets because their physical and chemical structures allow them to be inert when mixing with the intestinal bolus (Cao et al., 2003; Mateos et al., 2012). There has been a substantial variability in the results obtained when assessing the impact of dietary fiber in poultry nutrition due to differences in fiber type and diet formulation (Hetland et al., 2003; Jiménez-Moreno et al., 2009; Sadeghi et al., 2015), (Hetland and Svihus, 2001a). Both soluble and insoluble fibers have practical implications for the feed and monogastric animal industries,

especially with the current increase in the utilization of alternatives feedstuffs high in fibers which makes the understanding of the functional role of different DF in poultry nutrition a paramount step in the seeking for production efficiency.

2. Effect of dietary fiber in poultry diets

The unique ability of fiber to escape digestion and absorption provides the opportunity to regulate intestinal morphology (Rezaei et al., 2018; Sklan, Smirnov, and Plavnik, 2003), interact with nutrients of the digesta (Hetland et al., 2003), interact with intestinal microflora (Mahmood and Guo, 2020; Rehman et al., 2009), and modulate general digestive organ activity (Jorgensen et al., 1996; Sittiya et al., 2020), resulting in changes in nutrient utilization and growth performance. It has also been reported that different components of DF can modulate the physiological structure and functionality of the gastrointestinal tract differently (Dibner et al., 1996; Jorgensen et al., 1996; Sadeghi et al., 2015). All these changes present an overall modulation of the nutrient metabolism that might result in impacts on performance. The way dietary fibers exert their effects seems to be related with changes in morphology, organ growth, general nutrient digestibility, and microbiota. Such parameters will be discussed herein.

2.1 Intestinal morphology.

Poultry require a certain amount of DF for normal intestinal physiology functions to take place (Gonzalez-Alvarado et al., 2007). The mechanisms by which DF functions in the gastrointestinal tract depend in the chemical structure, particle size, and amount being used (Adibmoradi et al., 2016; Hetland and Svihus, 2001a; Holscher, 2017). Across poultry species there has been reported a rapid and relatively consistent intestinal responses to changes in DF resulting in modification of intestinal length, villus height, crypt depth as well as the passage rate

and size through different segments of the intestines (Cao et al., 2003; Chiou et al., 1996; Jin et al., 1994; Sklan et al., 2003). The improvements in villus height and overall epithelial cell arrangement have been regarded as desirable due to the potential increase in nutrient absorption. Such changes are, more often than not, seen when feeding fibers in most poultry species (Table 2.1). In a study (Sklan et al., 2003), it was shown that feeding isonitrogenous and isocaloric diets with increments of crude fiber from 2.8 to 9% to turkeys resulted in an increased in the number and size of villi in all sections of the small intestine with higher fiber-containing diets. Similar results have been reported in quails (Rezaei et al., 2018) fed 1.5% micronized wheat fiber which usually results in an increase in relative length of intestinal segments, villi height, villus thickness, and villi to crypt proportions. In geese, it was reported (Chiou et al., 1996) increases in villi height with inclusions of alfalfa, rice hulls or pectins; no changes with inclusion of barley hulls or cellulose; and reductions in villi height with inclusions of lignin. However, the inherent increment in nutrients for the maintenance of such tissues is generally ignored (Tejeda and Kim, 2020). In a study (Kluth and Rodehutscord, 2009), it was reported that inclusion of 8% cellulose to broiler diets resulted in higher crude protein and amino acid (i.e. Glu, Asp, and Thr) losses compared to diets fed 3% cellulose. It is important to bear in mind that such endogenous losses might not be from specific endogenous loses but also from dietary loses. However, there is a lack of research in this area.

There has been pointed out the increase in inevitable crude protein and amino acid endogenous loses in broilers fed high fiber levels (Kluth and Rodehutscord, 2009). Therefore, an important question to ask is whether improvements in villi height should be considered as an advantage because more villi height, in theory, more absorptive capacity; or should such improvements be considered a burden because more villi height leads to more cell turnover,

which might increase the requirements for amino acids important for intestinal functionality (i.e. threonine, aspartic acid, glutamic acid, proline). In such a case, adequate nutrient matrix modifications should be made to offset for such changes and ensure maximum growth.

2.2 Organ growth.

Poultry species have a characteristic digestive tract composed of beak, esophagus, crop, proventriculus, ventriculus (gizzard), small and large intestine. Proventriculus, gizzard, and the intestines play the role of digestion and absorption, thus, being the most influenced by dietary changes (Birger Svihus, 2014). The proventriculus is were HCl is secreted, but due to its small volume, the majority of mechanical digestion, *per se*, takes place in the gizzard (B. Svihus, 2011). Nevertheless, fiber inclusion affects proventriculus and gizzard in conjunction (**Table 2.1**). One important role of the gizzard is to regulate digesta particle size in the gastrointestinal tract (Hetland et al., 2004; Svihus, 2011) with the ability to sense and modulate the passage of feed from the upper digestive tract to the small intestine based on particle size. Factors such as fiber type and particle size are determinant factors that stimulate the muscular activity of the gizzard, resulting in increased size (Gonzalez-Alvarado et al., 2008). The normal retention of feed in the gizzard has been shown to be between half an hour to one hour which can increase up to two hours when structural (i.e. fiber) components are added to the diets (van der Klis et al., 1990). In an experiment (Amerah et al., 2009), it was reported that inclusion of 6% wood shavings increased the size of the proventriculus and gizzard while reducing the relative empty weight of the small intestine and increasing feed efficiency by 4.7%. Similarly, studies using oat hulls and soyhulls at 3% in the diet have shown to result in increased proventriculus and gizzard size and resulted in improved feed conversion (Gonzalez-Alvarado et al., 2007). The increase in particle size and fiber in the diet increases muscular activity of the gizzard as a consequence of the need

for particle size reduction, resulting in heavier weights as observed by different researchers in different poultry species (Amerah et al., 2009; Gonzalez-Alvarado et al., 2007; Rezaei et al,. 2018). The increase in the size of the proventriculus and gizzard is a logical result of an increased volume due to the slower passage rate of the almost-intact feed particles, which can only be solved by muscular grinding in the gizzard.

The presence of insoluble dietary fiber such as cellulose, lignin, and arabinoxylans can also modulate the size of the small intestine, pancreas, and ceca which can result in improvements of the total tract apparent retention of nutrients and feed efficiency as described by different researchers (Gonzalez-Alvarado et al., 2007; Hetland et al., 2003; Jiménez-Moreno et al., 2016; Kheravii et al., 2017). In an experiment (Sklan et al., 2003), the authors observed that turkey hens fed 6% and 9% crude fiber had a reduction of digestibility of crude protein, fat, and gross energy during the first 4 weeks of age which disappeared at the end of the eighth week. In fact, at the end of the experiment (week 14) such birds had an improved body weight compared to the control group (group fed 3% CF). Such changes were due to the prompt ability of the GI tract to compensate for changes in dietary fiber increasing the ability to use nutrients. One of the targets when using insoluble dietary fiber is to increase pancreatic secretions (i.e. amylases, lipases, proteases) that can improve substrate breakdown and subsequent release of nutrients. It has been reported that additions of insoluble fibers at 1% in diets of pullets can increase the relative weights of proventriculus, gizzard and liver and improve pancreatic proteolytic activity (Yokhana et al., 2016). Similarly, chickens fed 3% wheat bran have shown increased relative weights of gizzard, small intestine, and pancreatic amylase and trypsin activity that was correlated with increased nutrient digestibility (Shang et al., 2020). The presence of such indigestible carbohydrates (i.e. cellulose, arabinoxylans), and other indigestible plant components (i.e. lignin) upregulate

digestion activity as a means to compensate for the reduced hydrolysis of glycosidic bonds among molecules, resulting in an increased nutrient breakdown (digestibility) of others (i.e. starches, protein) (Hetland et al., 2004). Therefore, insoluble fiber with particle size bigger than 1.5 mm can help in the stimulation of digestive organ growth with potential changes in nutrient digestibility.

2.3 Nutrient digestibility.

In most poultry research, insoluble dietary fiber has been used as nutrient diluent due to the lack of enzymes to digest β 1-4, β 1-3, β 1-6 linkages found in such non-starch polysaccharides (Raza et al., 2019) which, have been regarded to impair performance when used in high amounts due to a slowing down and dilution of nutrient intake (Heywang 1950). As a consequence, commercial diets are generally formulated to contain a maximum of 2-3% CF (Choct, 2015). However, inclusion of specific insoluble fiber types such as cellulose at 3-5% in the diet has often proven to improve nutrient utilization. DF can also increase pancreas enzymatic activity and reverse peristalsis that can lead to increase in nutrient digestibility (Amerah et al., 2009; Hetland et al., 2003; Mateos et al., 2012). The reverse peristalsis causes bile salts to reach the gizzard where the bolus is being mixed with gastric secretions. This results in an improved fat emulsification, reducing the potential of fat droplets to coat nutrients and as a consequence nutrients are more readily hydrolyzed and absorbed (Hetland et al., 2004). However, the results obtained when using dietary fiber can be heavily impacted by the source of fiber and the formulation of iso-nitrogenous and iso-caloric diets (**Table 2.2**).

Inclusion of insoluble fibers such as cellulose and lignin from plant sources, at 3-5% in the diet, is commonly known to improve nutrient metabolism due to their ability to modulate

gastric secretions from the proventriculus and muscular activity from the gizzard (Sacranie et al., 2012; Svihus, 2011). The gizzard is a grinding organ equipped with both large and small muscles. The grinding is performed by larger muscles whereas smaller muscles are in charge of positioning the luminal contents for particle size reduction and gastric digestion. The movement of the digesta out of the gizzard is based on particle size, which is controlled by the small openings of the pylorus which functions as a sieve (Svihus, 2011). Regardless of the initial size, the organic feed components leaving the gizzard have a consistent particle size range (Hetland et al., 2004). It would follow that larger particles of DF will help in the retention of bolus in the upper portion of the gastrointestinal tract, slowing down the passage rate and increasing the exposure of feed components to HCl and enzymes from the proventriculus. This results in the accumulation of insoluble fiber in the gizzard, and increases the gastroduodenal reflux and subsequent digestibility of nutrients (Hetland et al., 2004; Sacranie et al., 2012). Insoluble dietary fiber has been shown to modulate (often times positively) digestion of starches (Amerah et al., 2009), fats (Jiménez-Moreno et al., 2009) and crude proteins (Cao et al., 2003) when added at 3-5% in the diet.

Soluble fibrous components of the diet such as pectins and arabinoxylans have been regarded to increase intestinal viscosity, reducing the absorption of nutrients (Silva et al., 2013) and modulating digesta passage rate that create environments full of substrates for microbial growth (Mateos et al., 2012; Tellez et al., 2014). Viscosity-forming soluble fibers such as β-glucans, pectins, and arabinoxylans have the ability to interact with water molecules (Chaplin, 2003) slowing down the passage rate in the small intestines reducing enzyme diffusion and subsequent substrate breakdown, increasing the free nutrients in the intestinal lumen, which favors pathogenic bacteria establishment that have been regarded to play a critical role in the competition for nutrient utilization with the host (Silva et al., 2013). In a study (Maisonnier et el.,

2010), apparent digestibility of lipids, protein, and metabolizable energy showed a linear decrease when feeding 0, 1, and 3g/kg of guar gum to broiler chickens decreasing feed efficiency by 4% when fed at 3g/kg in the diets. Inclusion of soluble fiber such as high-methylated pectins reduced feed efficiency up to 28% when provided in diets at 3% (Langhout and Schutte, 1996). Therefore, soluble viscous-forming fibers are undesirable at any levels in diets of broilers due to negative impacts in nutrient digestibility.

Two of the most prominent factors affecting digestion efficiency of nutrients in the presence of soluble fiber are solubility and fermentability because of their impact on passage rate in the small intestines and the fermentability in the hindgut, respectively (Dvir et al., 2000; Kheravii et al., 2018). Both of these factors are determined by the type of linkages and the amount of branching among sugar units that allows or prevents interactions with water molecules and/or potential bacterial break down (Chaplin, 2003). It is accepted that long β 1-4 chains, such in the case of cellulose, are poorly soluble whereas β 1-3 branches are highly soluble such in the case of β-glucans (Samaan, 2017). In poultry nutrition the term water-soluble carbohydrate has been erroneously interchanged with the term antinutritional fiber. Even though most of the soluble fibers have the ability to form viscosity in the presence of water, there is a small group of soluble fibers that do not. In fact, low-molecular weight carbohydrates such as oligosaccharides are regarded as prebiotics that facilitate the growth of beneficial bacteria from which Lactobacillus spp. and Bifidobacterium spp. have been targeted as beneficial for intestinal development (Ricke et al., 2020; Teng and Kim, 2018). Therefore, the hygroscopic properties of some oligo- and polysaccharides should not necessarily be directly associated with anti-nutritional factors.

The difference in how soluble and insoluble fiber affect intestinal passage rate relies on the site of action of each fiber type. When insoluble fiber is fed as particles bigger than 1.5 mm,

it can accumulate in the upper part of the gastrointestinal tract (i.e. gizzard and duodenum loop) where most of the bolus is mixing with enzymes and the mechanical grinding is taking place in the gizzard (Ferrando et al., 1987). While small (3-5%) additions of insoluble fibers can improve nutrient digestibility, extreme supplementation can interrupt normal digestion metabolism by the formation of coating structures that reduce the accessibility of digestive enzymes to nutrient (Jha et al., 2019; Walugembe et al., 2015); therefore, it is unclear how the threshold for excess DF should be defined. Type and source of fiber, as well as other parameters intrinsic to diet formulation, may influence this threshold. Finally, it is paramount to bear in mind that fiber should be used as a functional nutrient and not as a nutrient *per se*, and the adequate nutritional amendments should be made when using fibrous feedstuffs in terms of energy, protein and their ratios.

2.4. Dietary fiber and intestinal microflora activity.

After the bacterial inoculum introduced at hatch, the diet plays the most crucial role in determining the composition and density of the intestinal microflora (Yadav and Jha, 2019). As specific bacterial species have substrate preferences, it would follow that bacterial populations in the intestines are influenced by changing the diet (Apajalahti et al., 2004). The ceca is considered the main site of bacterial activity in the gastrointestinal tract in poultry and is, generally, the organ used for determination of bacterial populations in broilers (Baurhoo et al., 2007). The carbohydrate fraction is the most important dietary component regulating the intestinal microbial activity in broilers, particularly with regards to DF, which escapes digestion (Józefiak et al., 2010; Wagner and Thomas, 1978). The magnitude of the effects of the dietary carbohydrates depends on the type and amount of carbohydrate. Most data has indicated that water-soluble NSP are the most influential compounds, as these can be degraded to be utilized as substrate by intestinal

bacteria (**Table 2.3**) (Guillermo Tellez et al., 2014; Wagner and Thomas, 1978). These soluble components provide the energy for bacteria allowing them to use other nutrients (i.e. nitrogen) as substrates for the production of metabolites. It is clear that the presence of viscous-forming carbohydrates in the digestive tract have adverse effects on performance (Langhout and Schutte, 1996), but the presence of bacteria appears to aggravate the problem. In a study (Langhout, 1998), it was observed that germ-free broilers fed methylated citrus pectin were not strongly affected in terms of ileal digestibility of starch and energy compared to conventional broilers. Therefore, it is thought that the negative effects of water-soluble carbohydrate on performance and general metabolism in broiler is worsened by intestinal microflora and not only by intestinal viscosity.

Feed ingredients affect bacterial populations differently depending on the type and length of carbohydrates that are made of. In a study (Józefiak et al., 2010), it was observed that barley and rye tend to favor the development of pathogenic bacteria (i.e. Clostridium coccoides) and reduction of beneficial bacteria (i.e. Bifidobacterium sp.) when compared to groups containing enzyme addition. This difference can be explained by the fact that soluble fiber is generally associated with imbalances in microflora, favoring anaerobic pathogens that compete with the host for the uptake of nutrients (Lan et al., 2005; Mirzaie et al., 2012). Even though all viscous NSP are deemed as soluble, not all soluble NSP should be deemed as viscous (antinutritional). Groups of low-molecular weight compounds such as oligosaccharides and fructans are highly soluble and fermented by microbiota in the large intestine of broilers, and can be used to generate volatile fatty acids and other beneficial chemical compounds (Choct, 2015; G. Tellez et al., 2006). In fact, such low-molecular weight carbohydrates are frequently used as prebiotics to promote the growth of beneficial bacteria in the intestines (Dittoe, 2018). Soluble NSP such as β-glucans have been shown to positively alter the expression of immune genes associated with T helper type-1 cells,

resulting in downregulation of nitric oxide synthase, interleukins, and gross lesion severity in birds infected with Eimeria (Cox et al., 2010).

A balanced microflora in healthy broilers has the ability to produce a diverse number of metabolic end products including antigenotoxic compounds and short-chain fatty acids (SCFA) (Józefiak et al., 2004). There are different SCFA that can be synthesized in the ceca including acetic, propionic, and butyric acid (Eeckhaut et al., 2011). The type and quantity of fiber and other undigested dietary compounds reaching the posterior gut are the main factor determining the type of bacteria and the type of metabolite being produced. Among these metabolites, butyric acid has been regarded as the most beneficial SCFA due to its antimicrobial and anti-inflammatory properties (Place et al., 2005), as well as its use as an energy source by epithelial cells (Vernia et al., 2000). Fermentation of fiber in the gastrointestinal tract has been associated with increases in butyric acid, which may serve as a source of energy for enterocytes or as an antimicrobial for pathogenic bacteria (Namkung et al., 2011). Therefore, the production of butyric acid may lead to promotion of intestinal health.

2.5 Growth performance.

The growth performance is the sum of all the parameters aforementioned. In general, improvements in intestinal morphology and organ development can lead to increase nutrient absorption that will be reflected in enhanced performance (Sacranie et al., 2012; Sittiya et al., 2020; Yokhana et al., 2016). As is clear, different carbohydrates from dietary fiber can have different modes of action once ingested by the bird. Therefore, in order to make conclusions about the effect of fiber, there are different factors that need to be closely considered. Factors such as fiber source (i.e. soluble vs insoluble), particle size, level of inclusion, specie, age, physiological

status (i.e. laying hen vs broiler), dietary energy and protein (i.e. amino acids) levels, and duration of inclusion are among the most influential factors determining the effects of fibers on broiler diets (**Table 2.4**) (Amerah et al., 2009; Hetland and Svihus, 2001a; Jiménez-Moreno et al., 2009; Shakouri et al., 2009; Sklan et al., 2003; Tejeda and Kim, 2020). Most studies report changes in performance when insoluble fiber is included in the diets.

In general, inclusion of insoluble fiber as oat hulls, wood shavings, and soyhulls have been shown to increase the feed efficiency between 3-5% and increase body weight between 2-5% when included at 3-5% in the diet (Amerah et al., 2009; Gonzalez-Alvarado et al., 2007; Hetland et al., 2003). It is important to point out that many of the papers herein cited did not formulate isonitrogenous and isocaloric diets. This might be one of the reasons behind the differences observed in the results obtained when using dietary fiber. On the other hand, inclusion of soluble fiber such as high-methylated pectins reduced feed efficiency up to 28% when provided in diets at 3% (Langhout, 1998); soluble fiber such as guar gum decreases feed efficiency by 4% when fed at 0.3% in the diets (Maisonnier et al., 2010).Another reason is the difference in fiber type composition as shown in Table 2.5.

The insoluble portion of the plant cell wall is tri-dimensionally arranged in fibrillar polysaccharides such as cellulose, hemicellulose, and/or encrusting non-saccharide substances such as lignin (Hetland et al., 2004). Predominantly, insoluble fiber of the cell walls is associated with other polysaccharide matrices of pectic carbohydrates, conferring different structural and functional characteristics depending on their amounts (Keegstra, 2010). Because of this intrinsic chemical and structural organization, it is hard to separate soluble from insoluble NSP in feedstuffs and is important to understand both fractions individually and in conjunction when formulating diets for poultry species. The ratios of insoluble and soluble components can vary

based on grain type, cultivar, environmental conditions and other associated factors (**Table 2.5**). In general, water insoluble NSP contain long sequences of β-1,4 glycosidic units. The solubility of a polysaccharide is determined by the intramolecular (i.e. saccharide-saccharide interaction within molecule) and molecule-water interactions. For insoluble polysaccharides the intramolecular interactions are higher, including more hydrogen bonding. Insoluble fiber components include cellulose, hemicellulose, and lignin (Choct, 2015; Dhingra et al., 2012). Soluble fiber is found in association with insoluble fiber mainly as xyloglucan-cellulose, and xyloglucan-pectic polysaccharides (Keegstra et al., 1973) (**Table 2.5**). The tri-dimensional structure of soluble fiber is referred as matrix polysaccharides which includes mainly arabinoxylans, β-glucans and pectin (Hetland et al., 2004). The soluble carbohydrates, including oligosaccharides and polysaccharides, are the most influential in terms of growth performance, nutrient absorption modulation, and intestinal welfare. In general, water-soluble or partially water soluble NSP have β-1,4 glycosidic linkage backbones with β-1,3 linkages. The degree of solubility is associated with the degree of branching of the NSP molecule.

3. Applications of dietary fibers

Dietary fiber is an intrinsic component in cereal grains and oilseeds used in the formulation of broiler and poultry diets. Both, insoluble and soluble fiber components have direct effects in intestinal morphology, organ growth, nutrient utilization, and microflora modulation, at different extents. The results obtained when using dietary fiber relies on factors such as fiber type, inclusion level, particle size, and diet formulation. The insoluble fibers are regarded as functional nutrients because of their ability to scape digestion and modulate nutrient digestion and general intestinal parameters. Because of their insolubility, they have minimal or no effect on the intestinal microflora with significant effects in intestinal development and nutrient

digestibility when used on amounts between 3-5% in the diet. On the other hand, the group of soluble fibers has been regarded as antinutrients because of their hygroscopic properties and their ability to modulate intestinal functionality whether directly or indirectly through microbial changes. The presence of soluble fibers such as pectin and arabinoxylans can substantially impact the accessibility of the intestinal enzymes to substrates, resulting in lower nutrient release and subsequent nutrient digestibility. Nevertheless, it is important the emphasize that not all water-soluble fibers are antinutritional, low molecular weight water-soluble carbohydrates such as mannan-oligosaccharides, inulin, and other prebiotics play an important role in the modulation of intestinal microflora and potential immune response. Insoluble as well as soluble carbohydrates are found in cereal grains and oilseeds and, thus, is something poultry nutritionist have to deal with during diet formulation. To accurately determine the positive as well as the "unseen" negative effects of dietary fiber, it is important the accurate determination of fiber from feed ingredients with more adequate methodologies that may allow for the assessment of saccharides. Finally, to be able to make a correct use of fibrous feed ingredients is paramount to carry out experiments that would integrate the metabolic impact of dietary fiber on intestinal and organ development, nutrient digestibility, gene expression, and be able to introduce such corrections into the nutrient matrix.

REFERENCES

Adibmoradi, M., B. Navidshad, and M. F. Jahromi. 2016. The effect of moderate levels of finely ground insoluble fibre on small intestine morphology, nutrient digestibility and performance of broiler chickens. Ital. J. Anim. Sci. 15:310–317.

Ahsan, U., O. Cengiz, I. Raza, E. Kuter, M. F. A. Chacher, Z. Iqbal, S. Umar, and S. Çakir. 2016 Sodium butyrate in chicken nutrition: The dynamics of performance, gut microbiota, gut morphology, and immunity. W. Poult. Sci. J. 11:3981-3993.

Amerah, A. M., V. Ravindran, and R. G. Lentle. 2009. Influence of insoluble fibre and whole wheat inclusion on the performance, digestive tract development and ileal microbiota profile of broiler chickens. Br. Poult. Sci. 50:366–375.

Apajalahti, J., A. Kettunen, and H. Graham. 2004. Characteristics of the gastrointestinal microbial communities, with special reference to the chicken. W. Poult. Sci. J. 60:223–232.

Awad, W.A., E. Mann, M. Dzieciol, C. Hess, S. Schmitz-Esser, M. Wagner, and M. Hess. 2016. Age-related differences in the luminal and mucosa-associated gut microbiome of broiler chickens and shifts associated with Campylobacter jejuni infection. Front. Cell. Infect. Microbiol. 6:1-17.

Bailoni, L., S. Schiavon, G. Pagnin, F. Tagliapietra, and M. Bonsembiante. 2005. Quanti-qualitative evaluation of pectins in the dietary fibre of 24 foods. Ital. J. Anim. Sci. 4:49–58,

Baurhoo, B., L. Phillip, and C. A. Ruiz-Feria. 2007. Effects of purified lignin and mannan oligosaccharides on intestinal integrity and microbial populations in the ceca and litter of broiler chickens. Poult. Sci. 86:1070–1078.

Bedford, M. 2000. Removal of antibiotic growth promoters from poultry diets: Implications and strategies to minimise subsequent problems. W. Poult. Sci. J. 56:347–365,

Cant, J. P., B. W. McBride, W. J. Croom. 1996. The regulation of intestinal metabolism and its impact on whole animal energetics. J. Anim. Sci. 74:2541–2553.

Cao, B.H., X. P. Zhang, Y. M. Guo, Y. Karasawa, T. Kumao. 2003. Effects of dietary cellulose levels on growth, nitrogen utilization, retention time of diets in digestive tract and caecal microflora of chickens. Asian-Australas. J. Anim. Sci. 16:863–866.

Chaplin, M. F. 2003. Fibre and water binding. Proc. Nutr. Soc. 62:223–227,

Chiou, P.W.S., T. W. Lu, J. C. Hsu, B. Yu. 1996. Effect of different sources of fiber on the intestinal morphology of domestic geese. Asian-Australas. J. Anim. Sci. 9:539–550.

Cho, S.S., P. Samuel. 2009. Fiber Ingredients: Food Applications and Health Benefits. CRC press, Florida.

Choct, M. 2015. Fibre—Chemistry and Functions in Poultry Nutrition. LII Simp. Cient. Avic. 113–119.

Choct, M. 2009. Managing gut health through nutrition. Br. Poult. Sci. 50:9–15,

Cox, C.M., L. H. Sumners, S. Kim, A. P. Mcelroy, M. R. Bedford, R. A. Dalloul. 2010. Immune responses to dietary β-glucan in broiler chicks during an Eimeria challenge. Poult. Sci. 89:2597–2607.

Dhingra, D., M. Michael, H. Rajput, R. T. Patil. 2012. Dietary fibre in foods: A review. J. Food Sci. Technol. 49:255–266.

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

Dittoe, D.K., S. C. Ricke, A. S. Kiess. 2018. Organic Acids and Potential for Modifying the Avian Gastrointestinal Tract and Reducing Pathogens and Disease. Front. Vet. Sci. 5:216.

Dvir, I., R. Chayoth, U. Sod-Moriah, S. Shany, A. Nyska, A. H. Stark, Z. Madar, S. M. Arad. 2000. Soluble polysaccharide and biomass of red microalga Porphyridium sp. alter intestinal morphology and reduce serum cholesterol in rats. Br. J. Nutr. 84:469–476.

Eeckhaut, V., F. van Immerseel, S. Croubels, S. de Baere, F. Haesebrouck, R. Ducatelle, P. Louis, P. Vandamme. 2011. Butyrate production in phylogenetically diverse Firmicutes isolated from the chicken caecum. Microb. Biotechnol. 4:503–512.

Ferrando, C., P. Vergara, M. Jiménez, E. Goñalons. 1987. Study of the Rate of Passage of Food With Chromium-Mordanted Plant Cells in Chickens (Gallus Gallus). Q. J. Exp. Physiol. 72:251–259.

Fischer, M. M., A. M. Kessler, L. R. M. de Sá, R. S. Vasconcellos, F. O. Roberti Filho, S. P. Nogueira, M. C. Oliveira, A. C. Carciofi. 2012. Fiber fermentability effects on energy and macronutrient digestibility, fecal traits, postprandial metabolite responses, and colon histology of overweight cats. J. Anim. Sci. 90:2233–2245.

Glick, B., T. S. Chang, R. G. Jaap. 1956. The Bursa of Fabricius and Antibody Production. Poult. Sci. 35:224–225.

Gonzalez-Alvarado, J.M., E. Jimenez-Moreno, R. Lazaro, G. Mateos. 2007. Effect of type of cereal, heat processing of the cereal, and inclusion of fiber in the diet on productive performance and digestive traits of broilers. Poult. Sci. 86:1705–1715.

González-Alvarado, J.M., E. Jiménez-Moreno, D. G. Valencia, R. Lázaro, G. Mateos. 2008. Effects of Fiber Source and Heat Processing of the Cereal on the Development and pH of the Gastrointestinal Tract of Broilers Fed Diets Based on Corn or Rice. Poult. Sci. 87:1779–1795.

Hetland, H., M. Choct, B. Svihus. 2004. Role of insoluble non-starch polysaccharides in poultry nutrition. W. Poult. Sci. J. 60:415–422.

Hetland, H., B. Svihus. 2001. Effect of oat hulls on performance, gut capacity and feed passage time in broiler chickens. Br. Poult. Sci. 42:633–637.

Hetland, H., B. Svihus. 2001. Ileal starch digestibility in growing broiler chickens fed on a wheat-based diet is improved by mash feeding, dilution with cellulose or whole wheat inclusion. Br. Poult. Sci. 42:633–637.

Hetland, H., B. Svihus, A. Krogdahl. 2003. Effects of oat hulls and wood shavings on digestion in broilers and layers fed diets based on whole or ground wheat. Br. Poult. Sci. 44:275–282.

Heywang, B. W. 1950. High Levels of Alfalfa Meal in Diets for Chickens. Poult. Sci. 29:804–811.

Holscher, H. D. 2017. Dietary fiber and prebiotics and the gastrointestinal microbiota. Gut Microbes. 8:172–184.

Jacob, J. P., A. J. Pescatore. 2012. Using barley in poultry diets-a review. J. Appl. Poult. Res. 21:915–940.

Jaworski, N. W., H. N. Lærke, K. E. Knudsen, H. H. Stein. 2015. Carbohydrate composition and in vitro digestibility of dry matter and nonstarch polysaccharides in corn, sorghum, and wheat and coproducts from these grains1. J. Anim. Sci. 93:1103–1113.

Jha, R., J. M. Fouhse, U. P. Tiwari, L. Li, B. P. Willing. 2019. Dietary fiber and intestinal health of monogastric animals. Front. Vet. Sci. 6:48.

Jiménez-Moreno, E., A. De Coca-Sinova, J. M. González-Alvarado, G. Mateos. 2016. Inclusion of insoluble fiber sources in mash or pellet diets for young broilers. 1. Effects on growth performance and water intake. Poult. Sci. 95:41–52.

Jiménez-Moreno, E., J. M. González-Alvarado, A. González-Serrano, R. Lázaro, G. Mateos. 2009. Effect of dietary fiber and fat on performance and digestive traits of broilers from one to twenty-one days of age. Poult. Sci. 8: 2562–2574.

Jin, L., L. P. Reynolds, D. A. Redmer, J. Caton, J. D. Crenshaw. 1994. Effects of dietary fiber on intestinal growth, cell proliferation, and morphology in growing pigs. J. Anim. Sci. 72:2270–2278.

Johnson, L.A., P. J. White, R. Galloway. 2008. Soybeans. AOCS press, Urbana, IL, pp. 269–296.

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

Józefiak, D., A. Rutkowski, S. Kaczmarek, B. B. Jensen, R. M. Engberg, O. HØjberg. 2010. Effect of β-glucanase and xylanase supplementation of barley- and rye-based diets on caecal microbiota of broiler chickens. Br. Poult. Sci. 51:546–557,

Józefiak, D., A. Rutkowski, S. A. Martin. 2004. Carbohydrate fermentation in the avian ceca: A review. Anim. Feed Sci. Technol. 113:1–15.

Kaldhusdal, M., M. Hofshagen. 1992. Barley inclusion and avoparcin supplementation in broiler diets. 2. Clinical, pathological, and bacteriological findings in a mild form of necrotic enteritis. Poult. Sci. 71:1145–1153.

Kays, A. E., J. B. Morris, Y. Kim. 2006. Total and Soluble Dietary Fiber Variation in cyamopsis tetragonoloba (L.) taub. (guar) genotypes. J. Food Qual. 29:383–391.

Keegstra, K, K. W. Talmadge, W. D. Bauer, P. Albersheim. 1972. The structure of plant cell walls. Plant Physiol. 51:188–196.

Keegstra, K. 2010. Plant cell walls. Plant Physiol. 154:483–486.

Kheravii, S. K., N. K. Morgan, R. A. Swick, M. Choct, S. B. Wu. 2018. Roles of dietary fibre and ingredient particle size in broiler nutrition. W. Poult. Sci. J. 74:301–316.

Kheravii, S. K., R. A. Swick, M. Choct, S. B. Wu. 2017. Coarse particle inclusion and lignocellulose-rich fiber addition in feed benefit performance and health of broiler chickens. Poult. Sci. 96:3272–3281.

Kluth, H., M. Rodehutscord. 2009. Effect of inclusion of cellulose in the diet on the inevitable endogenous amino acid losses in the ileum of broiler chicken. Poult. Sci. 88:1199–1205.

Knudsen, K. E. 2014. Fiber and nonstarch polysaccharide content and variation in common crops used in broiler diets. Poult. Sci. 93:2380–2393.

Lan, Y., M. W. Verstegen, S. Tamminga, B. A. Williams. 2005. The role of the commensal gut microbial community in broiler chickens. Worlds. Poult. Sci. J. 61:95–104.

Langhout, D. J. 1998. The Role of the Intestinal Flora as Affected by Non-Starch Polysaccharides in Broiler Chicks. Ph.D. Thesis, Wageningen, Netherlands.

Langhout, D. J., J. B. Schutte. 1996. Nutritional Implications of Pectins in Chicks in Relation to Esterification and Origin of Pectins. Poult. Sci. 75:1236–1242.

Lloyd, A. B., R. B. Cumming, R. D. Kent. 1977. Prevention of Salmonella Typhimurium Infection in Poultry By Pretreatment of Chickens and Poults With Intestinal Extracts. Aust. Vet. J. 53:82–87.

Mahmood, T., Y. Guo. 2019. Dietary fiber and chicken microbiome interaction: Where will it lead to? Anim. Nutr., 6:1–8.

Maisonnier, S., J. Gomez, B. Carré. 2010. Nutrient digestibility and intestinal viscosities in broiler chickens fed on wheat diets, as compared to maize diets with added guar gum. Br. Poul. Sci. 1668:37–41.

Maki, J. J., C. L. Klima, M. J. Sylte, T. Looft. 2019. The microbial pecking order: Utilization of intestinal microbiota for poultry health. Microorganisms. 7:376.

Mateos, G.G., E. Jiménez-Moreno, M. P. Serrano, R. P. Lázaro. 2012. Poultry response to high levels of dietary fiber sources varying in physical and chemical characteristics. J. Appl. Poult. Res. 21:156–174.

McDevitt, R.M., J. D. Brooker, T. Acamovic, N. H. Sparks. 2006. Necrotic enteritis, a continuing challenge for the poultry industry. W. Poult. Sci. J. 62:221–247.

Mirzaie, S., M. Zaghari, S. Aminzadeh, M. Shivazad. 2012. The effects of non-starch polysaccharides content of wheat and xylanase supplementation on the intestinal amylase, aminopeptidase and lipase activities, ileal viscosity and fat digestibility in layer diet. Iran. J. Biotechnol. 10:208–214.

Nabizadeh, A. 2012. The effect of inulin on broiler chicken intestinal microflora, gut morphology, and performance. J. Anim. Feed Sci. 21, 725–734.

Namkung, H., H. Yu, J. Gong, S. Leeson. 2011. Antimicrobial activity of butyrate glycerides toward salmonella typhimurium and clostridium perfringens. Poult. Sci. 90:2217–2222.

Nguyen, N., M. Jacobs, J. Li, C. Huang, D. Li, D. M. Navarro, H. H. Stein, N. W. Jaworski. 2019. Technical note: Concentrations of soluble, insoluble, and total dietary fiber in feed ingredients determined using method AOAC 991.43 are not different from values determined using method AOAC 2011.43 with the AnkomTDF dietary fiber analyzer. J. Anim. Sci. 97:3972–3983.

NRC. 2001. Nutrient Requirements of Beef Cattle: Seventh Revised Edition, 2001, The national academies press, Washington, DC.pp. 134–142.

Oviedo-Rondón, E. O. 2019. Holistic view of intestinal health in poultry. Anim. Feed Sci. Technol. 250:1–8.

Owusu-Asiedu, A., J. F. Patience, B. Laarveld, A. G. Van Kessel, P. H. Simmins, R. T. Zijlstra. 2006. Effects of guar gum and cellulose on digesta passage rate, ileal microbial populations, energy and protein digestibility, and performance of grower pigs. J. Anim. Sci. 84:843–852.

Place, R.F., E. J. Noonan, C. Giardina. 2005. HDAC inhibition prevents NF-κB activation by suppressing proteasome activity: Down-regulation of proteasome subunit expression stabilizes IκBα. Biochem. Pharmacol. 70:394–406.

Podolske, J.,S. S. Cho, R. Gonzalez, A. W. Lee, C. Peterson. 2013. Rice hulls fiber: food application, physiological benefits, and safety. Cereal Foods World. 58:127–131.

Prosky, L. 2000. When is dietary fiber considered a functional food? BioFactors. 12:289–297.

Ratcliffe, M. J. H. 2002. B cell development in gut associated lymphoid tissues. Vet. Immunol. Immunopathol. 87:337–340.

Raza, A., S. Bashir, R. Tabassum. 2019. An update on carbohydrases: Growth performance and intestinal health of poultry. Heliyon. 5(2019)e01437.

Rehman, H., W. Vahjen, A. Kohl-Parisini, A. Ijaz, J. Zentek. 2009. Influence of fermentable carbohydrates on the intestinal bacteria and enteropathogens in broilers. W. Poult. Sci. J. 65:75–89.

Rezaei, M., M. A. Torshizi, H. Wall, E. Ivarsson. 2018. Body growth, intestinal morphology and microflora of quail on diets supplemented with micronised wheat fibre. Br. Poult. Sci. 59:422–429.

Ricke, S.C., S. I. Lee, S. A. Kim, S. H. Park, Z. Shi. 2020. Prebiotics and the poultry gastrointestinal tract microbiome. Poult. Sci. 99:670–677.

Riddell, C., X. Konga. 2017. The Influence of Diet on Necrotic Enteritis in Broiler Chickens. Am. Avian Pathol. 36:499–503.

Rossi, M., C. Corradini, A. Amaretti, M. Nicolini, A. Pompei, S. Zanoni, D. Matteuzzi. 2005. Fermentation of fructooligosaccharides and inulin by bifidobacteria: A comparative study of pure and fecal cultures. Appl. Environ. Microbiol. 71:6150–6158.

Sacranie, A., B. Svihus, V. Denstadli, B. Moen, P. A. Iji, and M. Choct. 2012. The effect of insoluble fiber and intermittent feeding on gizzard development, gut motility, and performance of broiler chickens. Poult. Sci. 91:693–700.

Sadeghi, A., M. Toghyani, and A. Gheisari. 2015. Effect of various fiber types and choice feeding of fiber on performance, gut development, humoral immunity, and fiber preference in broiler chicks. Poult. Sci. 94:2734–2743.

Samaan, R.A. 2017. Dietary Fiber for the Prevention of Cardiovascular Disease. Elsevier Inc., Los Angeles, CA, USA, pp. 35–59.

Shakouri, M.D., P. A. Iji, L. L. Mikkelsen, and A. J. Cowieson. 2009. Intestinal function and gut microflora of broiler chickens as influenced by cereal grains and microbial enzyme supplementation. J. Anim. Physiol. Anim. Nutr. (Berl) 93:647–658.

Shang, Q., D.Wu, H. Liu, S. Mahfuz, and X. Piao. 2020. The impact of wheat bran on the morphology and physiology of the gastrointestinal tract in broiler chickens. Anim. 2020, 10.

Shojadoost, B., A. R. Vince, J. F. Prescott. 2012. The successful experimental induction of necrotic enteritis in chickens by Clostridium perfringens: A critical review. Vet. Res. 43:1–12.

Silva, V.K., V. de Souza Morita, and I. C. Boleli. 2013. Effect of pectin extracted from citrus pulp on digesta characteristics and nutrient digestibility in broilers chickens. Rev. Bras. Zootec. 42:575–583.

Sittiya, J., K. Yamauchi, W. Nimanong, N. Thongwittaya. 2020. Influence of levels of dietary fiber sources on the performance, carcass traits, gastrointestinal tract development, fecal ammonia nitrogen, and intestinal morphology of broilers. Rev. Bras. Cienc. Avic. 22:1–8, doi:10.1590/1806-9061-2019-1151.

Sklan, D., A. Smirnov, and I. Plavnik. 2003 The effect of dietary fibre on the small intestines and apparent digestion in the turkey. Br. Poult. Sci. 44:735–740.

Svihus, B. 2014. Function of the digestive system. J. Appl. Poult. Res. 23:306–314,

Svihus, B. 2011. The gizzard: Function, influence of diet structure and effects on nutrient availability. W. Poult. Sci. J. 67:207–223.

Swanson, K.S., C. M. Grieshop, G. M. Clapper, R. G. Shields, T. Belay, N. R. Merchen, G. C. Fahey. 2001. Fruit and vegetable fiber fermentation by gut microflora from canines. J. Anim. Sci. 79:919–926.

Tejeda, O. J., W. K. Kim. 2020. The effects of cellulose and soybean hulls as sources of dietary fiber on the growth performance, organ growth, gut histomorphology, and nutrient digestibility of broiler chickens. Poult. Sci. 99:6828–6836.

Tellez, G., S. E.Higgins, A. M. Donoghue, B. M. Hargis. 2006. Digestive physiology and the role of microorganisms. J. Appl. Poult. Res. 15:136–144.

Tellez, G., J. D. Latorre, V. A. Kuttappan, M. H. Kogut, A. Wolfenden, X. Hernandez-Velasco, X., B. M. Hargis, W. G. Bottje, L. R. Bielke, O. B. Faulkner. 2014. Utilization of rye as energy source affects bacterial translocation, intestinal viscosity, microbiota composition, and bone mineralization in broiler chickens. Front. Genet. 5:339.

Teng, P.Y., W. K. Kim. 2018. Review: Roles of prebiotics in intestinal ecosystem of broilers. Front. Vet. Sci. 5:245.

Toghyani, M., S. B. Wu, R. A. Pérez-Maldonado, P. A. Iji, R. A. Swick. 2017. Performance, nutrient utilization, and energy partitioning in broiler chickens offered high canola meal diets supplemented with multicomponent carbohydrase and mono-component protease. Poult. Sci. 96:3960–3972.

Trowell, H. 1990. Ischemic heart disease and dietary fiber. Am. J. Clin. Nutr. 25:926–932.

van der Klis, J.D., M. W. Verstegen, W. De Wit. 1990. Absorption of minerals and retention time of dry matter in the gastrointestinal tract of broilers. Poult. Sci. 69:2185–2194.

Vernia, P., G. Monteleone, G. Grandinetti, G. Villotti, E. Di Giulio, G. Frieri, A. Marcheggiano, F. Pallone, R. Caprilli, and A. Torsoli. 2000. Combined oral sodium butyrate and mesalazine treatment compared to oral mesalazine alone in ulcerative colitis: Randomized, double-blind, placebo- controlled pilot study. Dig. Dis. Sci. 45:976–981.

Wagner, D. D., and O. P. Thomas. 1978. Influence of diets containing rye or pectin on the intestinal flora of chicks. Poult. Sci. 57:971–975.

Walugembe, M., J. C. Hsieh, N. J. Koszewski, S. J. Lamont, M. E. Persia, M. F. 2015. Rothschild, M.F. Effects of dietary fiber on cecal short-chain fatty acid and cecal microbiota of broiler and laying-hen chicks. Poult. Sci. 94:2351–2359.

Yadav, S., R. Jha. 2019. Strategies to modulate the intestinal microbiota and their effects on nutrient utilization, performance, and health of poultry. J. Anim. Sci. Biotechnol. 10:1–11

Yokhana, J.S., G. Parkinson, T. L. Frankel. 2016. Effect of insoluble fiber supplementation applied at different ages on digestive organ weight and digestive enzymes of layer-strain poultry. Poult. Sci. 95:550–559.

Table 2.1. Physiological response of different poultry species to dietary fiber.

Specie	Ingredient [1]	Effects [2]	% [3]	Age/Duration [4]	Reference
Broilers	Oat hulls, sugar beet pulp	↑ RW of proventriculus and ceca; oat hulls ↑ RW of the gizzard.	3%	1 day-old/21 days	[3]
Broilers	Sugar beet pulp and rice hulls	Sugar beet pulp ↑ RW of jejunum and ileum, whereas rice hulls ↓ RW; rice hulls ↑ jejunal villi height.	3%	1 day-old/42 days	[6]
Turkeys	Mix of sunflower meal and soyhulls	↑ villi height and morphology parameters inconsistently in duodenum, jejunum, and ileum.	3, 6, and 9% CF	1 day-old/98 days	[15]
Quail	Wheat fiber	↓ RW of the liver, ↑ RW and villi:crypt ratio of duodenum, jejunum, and ileum at 1.5%.	0, 0.5, 1, and 1.5%	1 day-old/28 days	[16]
Geese	Alfalfa, barley hulls, rice hulls, cellulose, lignin, or pectin	↑ in villi height with alfalfa meal, rice hulls or pectin and reductions with lignin.	Vary	14 days-old/28 days	[26]
Broiler	Soyhulls and cellulose	Soyhulls ↑ duodenal, jejunal, and ileal villus height.	2–8% CF	1 day-old/20 days	[27]
Broiler	Wood shavings	↑ RW of proventriculus and gizzard; ↓ RW of small intestine.	6%	1 day-old/21 days	[29]
Broilers	Oat hulls, soyhulls	↑ RW of proventriculus and gizzard; ↓ RW of small intestine.	3%	1 day-old/21 days	[22]
Broilers	Inulin	↑ villi height either at 0.5 or 1%.	0.5, 1%	1 day-old/42 days	[30]
Broilers	Oat and barley hulls	↑ RW of gizzard and of intestines.	15%	1 day-old/17 to 32 d-of-age	[31]
Broilers	Pectin and beet pulp	Pectin ↓ the liver weight.	1.5 and 3%	1 day-old/6–27 days	[32]

[1]Indicates the ingredient that was used as the main source of dietary fiber. [2] ↑ = increased; ↓ = decreased; RW = relative weight. [3] Indicates the net % of the ingredient added to the diet; when percent is followed by crude fiber (CF) indicates that the ingredient was added to achieve that level of crude fiber. [4] Indicates the age of the poultry specie when the experiment was started. Duration indicates the duration of the experiment.

Table 2.2. Impact of fiber type and amount on nutrient digestibility.

Specie	Ingredient [1]	Effects [2]	%	Iso-Nutrient[3]	Age/Length	Reference
Broilers	Oat hulls	↑ the TAR of dry matter, organic matter, nitrogen, ether extract, and amen.	3%	No	1 day-old/21 days	[3]
Broilers	Oat hulls	↑ starch digestibility.	10%	No	11 day-old/22 days	[13]
Broilers	Soyhulls and cellulose	Soyhulls ↑ amino acids digestibility.	2–8% CF	Yes	1 day-old/20 days	[27]
Broilers	Cellulose	9% ↑ starch digestibility.	6%	No	1 day-old/21 days	[29]
Broilers	Oat hulls	↑ TTAD of dry matter, nitrogen and ether extract digestibility.	3%	Yes	1 day-old/21 days	[22]
Broilers	Oat and barley hulls at 50:50, wt:wt; coarse and fine	↓ AMEn digestibility, and ↑ starch digestibility.	15%	No	1 day-old/18 to 32 d-of-age	[31]
Broilers	Oat hulls	10% oat hulls ↓ AMEn but ↑ starch digestibility.	4, 10%	No	7 day-old/14 days	[44]
Broilers	Guar gum	↓ AD of lipids, starch, protein, and AMEn at 1 and 3 g/kg.	1 or 3 g/kg diet	Yes	7 day-old/14 days	[45]
Broilers	Pectin from citrus pulp	↑ AME and AMEn with levels of pectin; quadratic ↓ in dry matter digestibility; ↓ in nutrient digestibility.	1, 3, 5%	Yes	1 day-old/31 days	[46]
Broilers	Cellulose	↑ Arginine, and Valine digestibility.	3, 8% CF	No	1 day-old/21 days	[33]

[1]Ingredient that was used as the main source of dietary fiber. [2] ↑ = increse; ↓ = decrease; TAR = total apparent retention; TTAD = total tract apparent digestibility; AD = apparent digestibility AMEn = nitrogen-corrected apparent metabolizable energy. [3] Isocaloric and isonitrogenous diets.

Table 2.3. Influence of dietary fiber on intestinal microflora in poultry species.

Specie	Ingredient [1]	Effects [2]	% [3]	Age/Duration [4]	Reference
Quail	Wheat fiber	No effects.	0, 0.5, 1, and 1.5%	1 day-old/28 days	[16]
Broilers	Inulin	↑ bifidobacteria and decrease E. Coli counts in cecal contents.	0.5, 1%	1 day-old/42 days	[30]
Broiler	Mix DDG and wheat	↑ Selenomonadales, Enterobacteriales, and Campylobacterales.	6 (starter) and 8% (grower)	1 day-old/21 days	[56]
Laying hen	Mix DDG and wheat	No changes.	6 (starter) and 8% (grower)	1 day-old/21 days	[56]
Broilers	Rye or pectin	Ileal segments had 2 or 3-log higher counts compared to control group.	4.50%	1 day-old/14 days	[62]

[1]Indicates the ingredient that was used as the main source of dietary fiber. DDG = dried distillers' grains. [2] ↑ = increase/improvement; ↓ = decrease/impairment. [3] Indicates the net % of the ingredient added to the diet; when percent is followed by crude fiber (CF) indicates that the ingredient was added to achieve that level of crude fiber. [4] Indicates the age of the poultry specie when the experiment was started. Duration indicates the duration of the experiment.

Table 2.4. Influence of dietary fiber type on growth performance.

Specie	Ingredient [1]	Effects [2]	% [3]	Iso-Nutrient [4]	Age/Duration	Reference
Broilers	Oat hulls, sugar beet pulp	Oat hulls ↑ daily ABW by 7.6%.	3%	No	1 day-old/21 days	[3]
Broilers	Sugar beet pulp	↓ FE by 9%.	3%	No	1 day-old/42 days	[6]
Broilers	Oat hulls	↑ FE by 3%.	10%	No	11 day-old/22 days	[13]
Broilers	Oat hulls	10% oat hulls ↓ FE by 6%.	4 and 10%	No	7 day-old/14 days	[14]
Turkey	Sunflower meal and soyhulls	6% CF ↑ 2.5% BW; 9% CF ↓ FE by 3.8%.	3, 6, 9% CF	Yes	1 day-old/98 days	[15]
Quail	Wheat fiber	↑ BW by 5% and ↑ FE by 5% at 1.5% in the diet.	0, 0.5, 1, and 1.5%	No	1 day-old/28 days	[16]
Broilers	Soyhulls and cellulose	↑ FE by 8% compared to cellulose.	2–8% CF	Yes	1 day-old/20 days	[27]
Broilers	Wood shavings	↑ FE by 4.7%.	6%	No	1 day-old/21 days	[29]
Broilers	Oat hulls, soyhulls	↑ FE by 3.8%.	3%	Yes	1 day-old/21 days	[22]
Broilers	Inulin	↑ BWG by 8% from 25–42 days when at 1% in the diet.	0.5, 1%	Yes	1 day-old/42 days	[30]
Broilers	Oat and barley hulls	Fine hulls ↓ FE by 4.7%; coarse ↑ BWG by 2%.	15%	No	1 day-old/17 to 32 days-of-age	[31]
Broilers	Guar gum	↓ FE by 4% when fed at 3 g/kg.	1 or 3 g/kg diet	Yes	7 day-old/14 days	[45]
Broilers	Pectin and beet pulp	Pectin ↓ BWG by 28% and FE by 28% when fed at 3%.	1.5 and 3%	Yes	1 day-old/6–27 days	[49]

[1]Indicates the ingredient that was used as the main source of dietary fiber. [2] ↑ = increase/improvement; ↓ = decrease/impairment; BW = body weight; ABW = average body weight; FE = feed efficiency; BWG = body weight gain. [3] Indicates the net % of the ingredient added to the diet; when percent is followed by crude fiber (CF) indicates that the ingredient was added to achieve that level of crude fiber. [4] Isocaloric and isonitrogenous.

Table 2.5. Fiber type and composition of common ingredients from tables 1–4.

Ingredient	IF [1], %	SF [1], %	Major NSP [2]	Structure-Linkages	Reference
Oat hulls	83.3	1.7	Cellulose/lignin	Glu β 1–4/β-O-4	[74]
Beet pulp	1.9–3	28	Uronic acid	β 1–4	[75]
Rice hulls	87.3	2.7	Cellulose/arabinoxylan /lignin	Glu β 1–4/β-O-4	[76]
Sunflower meal	11.3	3.9	Xylose/uric acid	β 1–4	[77]
Wheat fiber	44.9	7.6	Cellulose	Glu β 1–4	[75]
Alfalfa meal	46.7	7.9	Cellulose/lignin	Glu β 1–4/β-O-4	[78]
Barley hulls	20.3	9.8	Cellulose/hemicellulose/lignin	Glu β 1–4/β-O-4	[79]
Cellulose	97	2.3	Cellulose	Glu β 1–4	[80]
Pectin	0	65.4	Uronic acid	β 1–4	[80]
Soyhulls	49.3	13.3	Pectin/galacturonic acid	galacturonic acid 1α→4 linkages	[81]
Wood shavings	91.7	-	Cellulose/lignin	Glu β 1–4/β-O-4	[13]
Inulin [3]	-	>90	Fructose units	β 2–1	[30,82]
Guar gum	26	32	Mannose/galactose	β 1–4/1–6	[83]
DDGS [4]	25.5	3.4	Arabinoxylan	β 1–4	[84]
Wheat	9.3	1.9	Arabinoxylan	β 1–4	[84,85]
Rye	11	4.2	Arabinoxylan	β 1–4	[77]

[1]Indicates that the insoluble fiber (IF) and soluble fiber (SF) content was extrapolated using contents of acid detergent and neutral detergent fibers. [2] NSP = non-starch polysaccharides. [3] From the original source (seed endosperm or leaves). Authors don't mention the source of inulin; it is accepted that most inulin is fermented by intestinal microbial (soluble) [82]. [4] DDGS = Dried distillers' grains with solubles.

CHAPTER 3

THE EFFECTS OF CELLULOSE AND SOYBEAN HULLS AS SOURCES OF DIETARY FIBER ON THE GROWTH PERFORMANCE, ORGAN GROWTH, GUT HISTOMORPHOLOGY, AND NUTRIENT DIGESTIBILITY OF BROILER CHICKENS[1]

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Abstract

This study evaluated the effects of dietary fiber provided as purified cellulose (solka floc®, SF) or soybean hulls (SH) on the growth performance, organ growth, intestinal histomorphology, and nutrient digestibility. A total of 420 one-day old Cobb® male broilers were randomly assigned to seven dietary treatments and reared to 20 days of age in battery cages (n=6 replicates per treatment). The control group consisted of a simple corn and soybean-meal based diet. The six fiber treatments had increasing amounts of SF or SH to achieve 4, 6, and 8% crude fiber (CF). Chromium oxide was added as an indigestible marker at 0.3% in all treatment diets from 14 to 20 d for nutrient digestibility analyses. Weights for digestive organs were taken on d 20. Growth performance was measured weekly. Birds fed 4% SH diet had a higher d 20 body weight gain compared to those fed 8% CF regardless of fiber sources ($P = 0.0118$). Control and 4% SH groups had the best feed conversion ratio among the treatments at 7, 14, and 20 d ($P < 0.05$). SH-containing diets had heavier relative gizzard and intestine weights ($P < 0.001$). Birds fed 8% SH diets had the highest duodenal villi height among the treatments ($P < 0.001$). Birds fed control and 4% SH had the highest jejunal villi height among the treatments ($P < 0.001$). Birds fed 4% SF and 4% SH had the highest ileal villi height among the treatments ($P < 0.001$). Dry matter digestibility was higher in 6% SF compared to 8% SH ($P = 0.0105$). In general, birds fed high SH diets had higher amino acid digestibility ($P < 0.001$). In conclusion, the study suggests that fiber type and inclusion level are crucial factors regulating intestinal development, nutrient digestion, and growth performance.

Key words: broiler chicken, dietary fiber, digestibility, gut morphology, organ growth

Introduction

Dietary fiber is an intrinsic component in plant feedstuffs and varies in amount, structure, digestibility, and solubility depending on the origin (Hetland et al., 2004). Previous studies have reported that components of dietary fiber are associated with changes in growth performance (Sadeghi et al., 2015) and general modulation of the gastrointestinal tract (Owusu-Asiedu et al., 2006). These changes include alterations in villi height and crypt depth (Sklan et al., 2003), enzymatic activity and digestive organ size (Mateos et al., 2012), and nutrient digestibility (Owusu-Asiedu et al., 2006; Sigleo and Vahouny, 1984). The different chemical structures of the fiber found in feedstuffs lead to differences in physicochemical properties that influence digestibility and solubility and, therefore, nutrient utilization (Hetland et al., 2004).

Soluble fiber sources have been indicated to contain hygroscopic compounds (i.e. pectins, gums, and mucilages) with the ability to trap water and increase viscosity of the digesta, leading to changes in passage rate and nutrient absorption (Langhout et al., 2000; Owusu-Asiedu et al., 2006; Perera et al., 2019; Tellez et al., 2014). Insoluble fiber (i.e. cellulose, hemicellulose, and lignin) is thought to be inert in the sense that it does not interfere with nutrient absorption, but it actually accumulates in the gizzard, increasing the retention time of smaller particles and the digestibility of starches, fats and crude protein (Cao et al., 2003; Mateos et al., 2012). In general, it has been suggested that fibrous feedstuffs can be added to the diet at 3-5% without causing any negative effects in nutrient digestibility or growth performance of different poultry species (Cao et al., 2003; Jiménez-Moreno et al., 2009; Sklan et al., 2003; Amerah et al., 2009)

The accumulation of fiber in the gizzard actually increases the retention time of smaller particles, increasing digestibility of nutrients (Hetland et al., 2004). Due to the lack of consistency in results obtained when studying dietary fiber (Jiménez-Moreno et al., 2009;

Sadeghi et al., 2015), understanding of the functional role of dietary fiber in poultry nutrition grants further investigation regarding roles of fiber inclusion level and type. Therefore, the objective of this study was to evaluate the effects of purified cellulose (solka floc®) or soybean hulls as sources of dietary fiber on the growth performance, organ growth, gut morphology, and nutrient digestibility of broiler chickens.

Material and methods

General Procedures

The experiment was approved by the Institutional Animal Care and Use Committee (IACUC) of the University of Georgia (Athens, Georgia, United States). A total of 420, one-day-old Cobb500™ broiler chicks (Cobb x Cobb) were distributed in a complete randomized design with seven dietary treatments and six replicates of 12 birds each. The inclusion level of CF from two fiber sources (cellulose and soyhulls) was considered the main factor. The chicks were allocated in 42 cages equipped with one drinker and one feeder, providing *ad-libitum* access to water and mash feed from 1 to 20 d of age. Temperature and lighting program followed the recommendation of Cobb Broiler Management Guide (Cobb-vantress 2018).

Dietary Treatments

The diets were corn and soybean meal-based and formulated to meet the nutrient requirements specified by Cobb500 performance and nutritional guide (Cobb-Vantress 2018). Diets were provided as mash during the entire rearing period (0-20 d). All diets were isonitrogenous and isocaloric and are shown in Table 3.1. Control was a corn-soybean meal-based diet containing 2% CF. Control diet was used as a basal diet to which purified cellulose (**SF**: 99% cellulose, Solka floc®, Skidmore, Schollcraft, MI) was added as a source of CF by replacing an inert filler (sand) to achieve 4, 6, and 8% CF (4% SF, 6% SF, and 8% SF) in the

diets. The rest three diets were formulated using increasing amounts of soyhulls (**SH**) to achieve 4, 6, and 8% CF (4% SH, 6% SH, and 8% SH). The nutrient matrix composition used for soyhull diets was taken from (Barros-Dourado et al., 2012). For ileal nutrient digestibility determination, chromic oxide (Cr_2O_3, Sigma Aldrich, St. Louis, MO) was added at 0.3% as an indigestible marker to all diets from 14-20 d.

Insert Table 3.1

Growth Performance and Organ Weights

The birds and feed were weighed weekly to determine mortality-corrected body weight gain (BWG), mortality-corrected feed intake (FI), and mortality-corrected feed conversion ratio (FCR). Mortality was recorded daily. On d 20, two average birds per cage were euthanized, and gizzard, pancreas, liver, duodenum, jejunum, ileum, and ceca were weighed to determine the relative organ weight.

Intestinal Morphology.

On d 20, samples from the duodenum, jejunum and ileum (~ 2cm long) were collected from one average bird per replicate cage (n = 6 per treatment). Intestinal samples were collected and stored in 10% neutral-buffered formalin and left in solution for a minimum period of 48 hours for tissue fixation. During slide preparation, the tissues were dehydrated in increasing amounts of ethanol, diaphanized in dimethylbenzene, and fixed in paraffin. Subsequently, tissue sections with a thickness of 4-µm on slides were stained using Hematoxylin and Eosin (H&E) procedures. Pictures were taken using a light microscope (10x eyepiece and 1.6x magnification; Leica DC500 camera, Leica Mycrosystems Inc., Buffalo Groove, IL). Measurements for villi

height and crypt depth were taken using ImageJ software (Image Processing and Analysis in JAVA – ImageJ 1.52r, National Instituted of Health).

Nutrient Digestibility.

On d 20, five birds per replicate cage were euthanized, and ileal digesta were collected from two-thirds of the distal ileum (from Meckel's diverticulum to about 1 inch anterior to ileocecal junction). The digesta samples were pooled and dried for analyses of energy, crude protein, and amino acids. The chromium oxide concentration was measured in duplicate according to Dansky and Hill (1952), and gross energy was evaluated in duplicate using a bomb calorimeter (IKA Calorimeter C1, IKA Works Inc., Wilmington, NC) at the University of Georgia. The crude protein (N × 6.25) and amino acids were analyzed at the Chemical Laboratories at the University of Missouri-Columbia. The apparent ileal digestibility (AID) of apparent metabolizable energy (AME), crude protein, amino acids, and dry matter were calculated using the following equation:

$$AID, \% = 100\left[1 - \left(\frac{Cr_{feed}}{Cr_{dig}}\right) \times \left(\frac{Nutrient_{dig}}{Nutrient_{feed}}\right)\right]$$

where Cr_{feed} and Cr_{dig} are the chromium dioxide in feed and ileal digesta, respectively; and $nutrient_{dig}$ and $nutrient_{feed}$ are the nutrient in ileal digesta and feed, respectively.

Statistical analyses.

Dietary fiber level was used as the fixed effect in the model. Pen was used as the experimental unit for growth performance and nutrient digestibility; bird was used as the experimental unit for organ growth and intestinal morphology. Data were analyzed using one-way Analysis of Variance by the following model:

$$Y_{ij} = \mu + \alpha_i + \varepsilon_{ij}$$

where Y_{ij} represents the value for each random variable; μ is the overall mean; α_i are the fixed factor level effects corresponding to the ith treatment such that $\Sigma\alpha_i=0$; and the random errors ε_{ij} are identically and independently normally distributed with a mean 0 and a variance σ. All statistical procedures were performed using SAS University Edition (SAS Institute, 2020). In case of significant differences, means were separated using the Tukey's test HSD option. For all hypothesis tests, statistical significance was considered at $P < 0.05$.

Results

Growth performance and organ weights.

The results for growth performance are shown in Table 3.2. Birds fed 4% SH diet had a higher d 20 body weight gain compared to those fed 8% CF regardless of fiber sources ($P = 0.012$). No statistical differences were observed in either BWG nor FI on 7 and 14 d ($P > 0.05$) among the treatments; however, 4% SH group had the highest BWG and FI during the entire study. Moreover, FCR of birds fed 4% SH diet had the lowest FCR among the treatment during the entire study. Group fed 4% SH group had significantly lower FCR compared to 8% SF or 8% SH on 7, 14, or 20 d ($P < 0.05$). Group fed 4% SH was heavier than the group fed 4% SF; however, the contrary was true in groups fed 6% SH and SF, respectively. The FCR of 4% SH was lower than the group fed 4% SF. However, the FCR of 6% and 8% SH was lower than those fed SF. There were no significant differences in BWG, FI and FCR between control and 4% SH groups ($P > 0.05$).

The results for relative organ weights and organ relative weights are shown in Table 3.3. The livers from birds fed the control diet were heavier than those fed 6 and 8% SH diets ($P = 0.001$). However, relative weight of the liver was not statistically different among different

dietary treatments ($P = 0.183$). The relative weight of the gizzard was higher in birds fed 6% SH diet compared to control, 4% SF, and 6% SF groups ($P < 0.001$), whereas birds fed 8% SH had significantly higher than those fed 4 and 6% SF. The relative weights of the jejunum and ileum were higher for birds fed 8% SH diet compared to the rest of treatments, except for 6% SH ($P < 0.001$). Birds fed 6% SH had significantly higher the relative weights of the jejunum and ileum compared to control, 4% SF or 6% SF.

Intestinal histomorphology.

The results for intestinal histomorphology are shown in Table 3.4. Birds fed 8% SH diet had the highest duodenal villi height among the treatments and significantly higher than those fed SF and SH groups ($P < 0.001$). The shortest duodenal villi height was observed in 6% SF-fed birds. No statistical differences were observed for duodenal crypt depth among the treatments ($P = 0.066$). Birds fed control and 4% SH diets had the highest jejunal villi height among treatments ($P < 0.001$). Birds fed 8% SH had significantly higher villi height than those fed 6% SH. The jejunal crypt depth of 4% SH-fed birds was significantly higher than those of 6% SF and 6% SH groups ($P = 0.008$). Birds fed 4% SF and 4% SH had significantly higher ileal villi height compared to the other treatments ($P < 0.001$). Ileal villi height of 6% SH group was significantly higher than that of 8% SF group. The ileal crypt depth of 4% SH group was significantly higher than those of 6% SF and 6% SH groups. No statistical differences were observed in duodenal or jejunal villi:crypt ratio ($P > 0.05$). Birds fed 6% SH diet had higher villi:crypt ratio than the control, 6% SF, and 8% SH groups ($P < 0.001$).

Insert Table 3.2

Nutrient digestibility.

Results for nutrient digestibility are shown in Table 3.5. Dry matter digestibility was higher in 6% SF compared to 8% SH diets ($P = 0.0105$). No differences in crude protein or AME digestibility were observed among treatments ($P > 0.05$). Birds fed 8% SH had the highest methionine digestibility among treatments ($P < 0.001$), and those fed 6 and 8% SH had the highest threonine digestibility among the treatments ($P < 0.001$). No differences in lysine, serine, and tryptophan digestibility were observed among the treatments ($P > 0.05$). In general, feeding 6 and 8% SH resulted in better digestibility of dietary essential amino acids (except lysine and tryptophan) and dietary non-essential amino acids (except serine) ($P < 0.05$). Birds fed 6% SH diet had significantly higher digestibility of valine, isoleucine, tyrosine, phenylaniline, histidine, arginine, aspartate, glutamate, proline, glycine, alanine, or cysteine compared to control, 6% SF, 8% SF or 4% SH ($P < 0.05$). The digestibility of isoleucine, leucine, tyrosine, phenylaniline, or aspartate in birds fed 8% SH was significantly higher than control, 6% SF, or 4% SH ($P < 0.05$). However, there was no significant difference in amino acid digestibility between 6 and 8% SH groups.

Insert Table 3.3

Insert Table 3.4

Insert Table 3.5

Discussion

Growth performance and organ weights.

Despite the fact that the diets were formulated with the same nutrient content, there were differences in growth performance among the treatments. Control and 4% SH groups had better FCR at 7, 14, and 20 d compared to 8% SH-fed group. However, no differences were observed between the 4% and 6% SF groups which indicates that insoluble fibers are inert when given in small amounts. The FCR of 4% SH was lower than the group fed 4% SF. However, the FCR of 6% and 8% SH similar to those fed SF, respectively. The group fed 4% SH was heavier than the group fed 4% SF the contrary was true in groups fed 6% SH and SF, where SF had heavier weights. There was a 5% improvement in BWG in 4% SH-fed birds compared to the control group on d 20. These results indicate that minimum amounts (4%) of dietary fibers are necessary to maximize growth performance in young broilers as indicated by other reports (Jiménez-Moreno et al. 2009). In a study, Gonzalez-Alvarado et al., (2007) reported a 5% increase in BWG and 2% improvement in FCR of broilers fed either 3% oat hulls or soyhulls compared to the control group. In the current study, the results obtained from feeding 4% CF were different for SH and SF despite the fact that both diets were formulated to be isonitrogenous and isocaloric, indicating that fiber type is a determinant factor in growth performance of broilers. On d 20, the BWG of birds fed 4% SH was 9% higher and the FCR was 8% lower (better) than those birds fed 4% SF diets. These results from the current study are in agreement with a study (Jiménez-Moreno et al. 2009) reporting that the inclusion of 3% oat hulls in rice-soy protein concentrate based diets improved BWG and FCR in broilers. Finally, inclusion of 8% CF either with SF or SH had adverse effects in BWG and FCR on 20 d of age. These results from the current experiment are in agreement with González-Alvarado et al., (2008) who reported 3-4%

crude fiber an adequate amount to be used in broilers. There are different reasons why inclusions of crude fiber higher than 4% may cause reduction in growth performance, especially when including soluble dietary fibers. The body weights and FCR was similar in groups fed 4% and 6% SF which points out that insoluble fibers tend to have an inert role in the gastrointestinal tract when provided in small amounts as described by Hetland et al., (2004). However, when given in larger amounts (higher than 8%), they can interrupt nutrient absorption resulting in decreased performance (Cao et al., 2003) as observed when feeding 8% SF in the current experiment. The presence of larger amounts of dietary fiber in the gastrointestinal tract increases organ size (i.e. gizzard, intestines) as a way to offset the increase of the volume (i.e. bulky diets) of feed moving through the intestines (González-Alvarado et al., 2008; Hetland et al., 2004; Rezaei et al., 2018; Svihus, 2011). These changes in organ growth may also increase maintenance requirements associated with increases in tissue synthesis and protein turnover, leading to more nutrients being directed toward maintenance of such tissues and less toward muscle protein accretion and growth performance (Nyachoti et al., 2000) even when adequate nutrient absorption is taking place in the gastrointestinal tract. Additionally, because of the bulkiness of diets containing fibrous components (i.e. SH and SF), increasing feed intake to compensate for such changes in nutrient partitioning does not seem to be possible; that might be why we observed the same FI but differences in BW and FCR when feeding 8% CF in isonitrogenous and isocaloric diets.

In the present study, it was observed that, except for liver weights, gross organ weights did not differ among treatments. Control group had heavier gross liver weights compared to 6 and 8% SH groups. Generally, body conformation is associated with internal organ size, with some variations in genetic lines (Kokoszyński et al., 2017); therefore, since control-fed group had heavier BW, it is logical to think that such BW is associated with heavier livers even though

relative (%) weights decrease overtime. This may also be associated with the negative impact of dietary fiber on fat digestibility. The current results are in agreement with González-Alvarado et al., (2008) who reported that the gross organ weights of birds fed 3% soyhulls did not differ statistically from the control group. However, in the current study it was observed that when body weight was considered, the relative organ weights differed among the treatments. Similarly, Sadeghi et al., (2015) reported that different fiber sources are effective in stimulating intestinal and organ growth. The addition of CF with SH had more remarkable effects on gizzard and small intestine relative weights. Birds fed 6% and 8% SH had heaver relative weight of the gizzard compared to control, 4%, and 6% SF diets. In general, 6 and 8% SH diets had heavier relative jejunum and ileum weights compared to the rest of treatments. The current results indicate how fiber type can play a crucial role in the development of digestive organs; compositions of different fiber sources may be attributed to changes in growth performance and organ development. Soybean hulls have hemicellulolytic (insoluble) and pectin (soluble) carbohydrates (Stein et al., 2008). The mixture of these soluble and insoluble carbohydrates in soyhulls might alter the gastrointestinal tract and digestive organs differently compared to purified cellulose which is a simple form of fiber. Finally, inclusion of dietary fiber in the form of soyhulls modulates organ growth, especially the gizzard and the different portions of the small intestine differently than cellulose. Similar results were reported by Chiou et al., (1996) when supplementing fiber in the form of alfalfa, barley, rice hulls, cellulose, lignin, or pectin.

Intestinal histomorphology.

Duodenal villus height was higher for the 8% SH-fed group compared to the rest of treatments, except for the control group. The groups fed 4 and 6% SH or SF, respectively, had similar results. The major differences were observed between 8% SH and SF groups. This might

be associated with the stimulation of intestinal development caused by the increase in intestinal reflux in the upper intestinal tract as observed in other experiments (Sacranie et al., 2012). In the current study, control and 4% SH groups had the highest jejunal villi height among the treatments. These results are in agreement with Praes et al., (2011) who observed that laying hens fed 7.5% soyhulls had an improvement in duodenal and jejunal villus height compared to other fiber sources; however, they did not observe differences in performance as seen in the current study. The ileal villus height and depth were higher for 4% SF and 4% SH groups compared to the rest of treatments. Dietary fiber is a critical factor affecting intestinal morphology as observed in this experiment and as reported by other authors in different poultry species (Hetland et al., 2003; Sklan et al., 2003; Chiou et al., 1996; González-Alvarado et al., 2008; Rezaei et al., 2018; Sadeghi et al., 2015). In the current study, SH-containing diets had a more pronounced effect in intestinal morphology compared to SF diets. According to Stein et al., (2008), SH contains 50% hemicellulose, 30% pectin, and 20% cellulose. The mix of different types of fibers appears to have a marked effect in intestinal morphology. Finally, there is a clear drop in the ileal and jejunal villus height in the 6% and 8% SH groups, which indicates that 4% CF as SH is adequate for stimulating intestinal villus growth in young broilers. Similarly, Sadeghi et al., (2015), reported that broilers fed sugar beet pulp at 3% in the diet had shorter jejunal and ileal villus height compared to the control and rice hull-fed groups. The same authors reported that sugar beet pulp contains 47% soluble carbohydrates (non-fiber carbohydrates) which points out that the presence of soluble fibers reduce villus growth. As previously mentioned, SH contains 30% soluble carbohydrates (i.e. pectins), therefore, higher inclusions of such water-soluble carbohydrates reduce villus height in the jejunum and ileum which might be

associated to the lack of "abrasive stimulus" that is generally seen in such fibers compared to insoluble fibers (Rezaei et al., 2018).

Nutrient digestibility.

Dry matter apparent digestibility was 9% lower for 8% SH-fed birds compared to 6% SF-fed group. The SF (cellulose) is a source of insoluble fiber (cellulose), whereas SH contains both soluble and insoluble fiber components. Cellulose and other insoluble fibers such as rice hulls and wood shavings act as inert materials affecting the gut functions and modulation of nutrient digestion that are often associated with improvements in nutrient digestion (Hetland et al. 2003). On the other hand, the viscous components of soluble fibers have been reported to reduce the coefficients of apparent digestibility of dry matter. Silva et al., (2013) reported that broilers fed pectin in increasing amounts from 10 to 50 g/kg had a quadratic and a linear response in the starter and grower phase, respectively; increase in pectin resulted in lower digestibility of dry matter which is similar to the results from the current study. Another study by Shakouri et al., (2009) reports that birds fed grains containing soluble and viscous NSP had a lower apparent digestibility of dry matter which can be attributed to the soluble portion of the fiber components. In the present study, despite the reduction in dry matter digestibility, the apparent digestibility of crude protein and ME did not differ among the treatments. Similarly, Hetland and Svihus (2001) observed no differences in AME_n in broiler fed 3% oat hulls as a fiber source; however, adding 10% oat hulls reduced AME_n. Unlike the results in the present study, Sklan et al. (2003) reported lower digestibility of crude protein, fat, and gross energy of turkeys fed 8-9% CF in diets where sunflower meal was used as the main source of dietary fiber. The lack of agreement can be associated to differences in fiber type, amounts, and specie-related differences.

Dietary fiber had significant effects in amino acid digestibility. The sum of essential amino acids (EAA) shows that groups fed 6% and 8% SH had higher total EAA digestibility. In general, inclusion of dietary fiber as 6 or 8% SH improved digestibility of all dietary-essential (except lysine and tryptophan) and dietary non-essential (except serine) amino acids. Digestibility for 6% and 8% SF groups was relatively constant compared to the groups fed SH. The sum of non-essential amino acids (NEAA) was similar among the groups. Finally, the EAA: NEAA ratio was close to 1.06 for all the treatments. Interestingly, 4% SH group had increased villi height but decreased amino acid digestibility and showed better BW and FCR compared to 8% SH group. On the other hand, 8% SH group had higher amino acid digestibility and worse BW and FCR. The relative weights of gizzard, jejunum, and ileum were higher for 8% SH group, which indicates the possibility that more nutrients are being directed toward maintenance of such organs (i.e. protein synthesis and turnover) and the nutrient partitioning is different between 8% and 4% SH group, resulting in changes in muscle protein accretion (i.e. growth performance). In addition, the lower dry matter digestibility in birds fed higher levels of SH (6, and 8%) seems to be compensated by an increase in amino acid digestibility. This indicates that birds have the ability to modulate the structure of the gastrointestinal to compensate for differences in dietary fiber components. Sadeghi et al., (2015) reported that changes in intestinal structure when broilers are exposed to 30 g/kg sugar beet pulp as soluble fiber, are part of an adaption mechanism to the lower diffusion rates of nutrients. Interestingly, the amino acid digestibility of 6 and 8% SH diets in the current study were different from that of SF diets (6, and 8%) which resulted in lower amino acids digestibility. Cao et al., (2003) reported that laying hens had a lower nitrogen digestibility and absorption when fed 10% cellulose. The inclusion of soyhulls in the diets of broilers had more profound effects in intestinal histomorphology and organ growth

that could be linked with improvement in amino acid digestibility. Improvement in amino acid digestibility, however, appears to be associated with an increase in nutrients requirements for maintenance of heavier digestive organs (i.e. gizzard, jejunum, and ileum) due to the presence of dietary fiber. Such changes in nutrient partitioning can be able to reduce muscle protein accretion and subsequent growth performance (Nyachoti et al. 2000). Fiber components, especially those water soluble, that escape digestion and absorption can also serve as substrate to intestinal bacteria that can synthesize short chain fatty acids (i.e. propionate, acetate and butyrate) that have been shown to have functional roles such as antimicrobial, a source of energy source, and intestinal immunomodulators (Fernández-Rubio et al. 2009; Liu et al. 2018) which can help in the improvement of the gastrointestinal tract, sometimes resulting in increased digestibility of amino acids as reported by Kaczmarek et al., (2016). Finally, in the current study, there were no differences in glucose and amino acid transporters (data not shown); however, this does not limit the probability of differences in the transporter proteins. Other authors have indicated the potential for improvement of protease activity when fibrous materials are added to diets of broilers (Hetland et al. 2004), but it is important to establish a balance between enzymatic activity stimulation, organ growth, and protein synthesis and turnover that can be regulated by dietary fiber inclusion to broiler diets in order to optimize performance. Therefore, further research is granted to better understand the role of dietary fiber on nutrient absorption and utilization.

Conclusions

Different fiber types and inclusion levels are determining factors in growth performance, and intestinal development and functionality. In the present study, 4% SH had 5% improvement in BWG compared to the control group and 9% and 8% improvement in BWG and FCR on d 20,

respectively, compared to the 4% SF group. Based on the results from the present study, it is concluded that CF can be added into broiler diets with SH at a level of 4% without having adverse effects in performance of broiler chickens under isonitrogenous and isocaloric dietary conditions. And even though 6% fiber can improve amino acid digestibility, growth was not favored with such fiber levels which might be associated with an increase of nutrient requirements for maintenance of a higher epithelial cell turnover. In summary, fiber type and inclusion level are crucial factors regulating growth performance, intestinal development, and nutrient digestion, and further research is granted to understand how different fiber components can affect broiler performance from a physiological and nutritional perspective. This will provide us a pathway by which we may be able to formulate cost-effective diets with inexpensive fibrous feedstuffs.

References

Amerah, A. M., V. Ravindran, and R. G. Lentle, R. 2009. Influence of insoluble fibre and whole wheat inclusion on the performance, digestive tract development and ileal microbiota profile of broiler chickens. Br. Poult. Sci. 50:366–375.

Barros Dourado, L. R., L. A. Fonseca-Pascoal, N. K. Sakomura, F. G. Perazzo-Costa, and D. Biagiotti. 2012. Soybeans (Glycine max) and Soybean Products in Poultry and Swine Nutrition.

Cao, B. H., X. P. Zhang, Y. M. Guo, Y. Karasawa, and T. Kumao. 2003. Effects of dietary cellulose levels on growth, nitrogen utilization, retention time of diets in digestive tract and caecal microflora of chickens. Asian-Austral. J. Anim. Sci. 16:863–866.

Chiou, P. W. ., T. W. Lu, J. C. Hsu, and B. Yu. 1996. Effect of different sources of fiber on the intestinal morphology of domestic geese. As.-Aus. J. An. Sci. 9:539–550.

Cobb-vantress. 2018. Broiler Management Guide. Accessed January 2020.

Cobb-Vantress. 2018. Broiler performance & nutrition supplement Cobb500. Accessed January 2020. Cobb Vantres.Com, 14. Retrieved from http://www.cobb-vantress.com/docs/default-source/cobb-500-guides/cobb500-broiler-performance-nutrition-supplement-(english).pdf.

Fernandez-Rubio, C., C. Ordonez, J. Abad-Gonzalez, A. Garcia-Gallego, M. Pilar-Hondubia, J. Jose-Mallo, and R. Balana-Fouce. 2009. Butyric acid-based feed additives help protect broiler chickens from Salmonella Enteritidis infection. Poult. Sci. 88:943-948.

Gonzalez-Alvarado, J. M., E. Jimenez-Moreno, R. Lazaro, and G. G. Mateos. 2007. Effect of type of cereal, heat processing of the cereal, and inclusion of fiber in the diet on productive performance and digestive traits of broilers. Poult. Sci. 86:1705-1715.

Gonzalez-Alvarado, J. M., E. Jimenez-Moreno, D. G. Valencia, R. Lázaro, and G. G. Mateos. 2008. Effects of fiber source and heat processing of the cereal on the development and ph of the gastrointestinal tract of broilers fed diets based on corn or rice. Poult. Sci. 87:1779–1795.

Hetland, H., and B. Svihus. 2001. Effect of oat hulls on performance, gut capacity and feed passage time in broiler chickens. Brit. Poult. Sci. 42: 633–637.

Hetland, H., B. Svihus, and Å. Krogdahl. 2003. Effects of oat hulls and wood shavings on digestion in broilers and layers fed diets based on whole or ground wheat. Brit. Poult. Sci. 44:275–282.

Hetland, H., M .Choct, and B. Svihus. 2004. Role of insoluble non-starch polysaccharides in poultry nutrition. W. J. Poult. Sci. 60: 415–422.

Kaczmarek, S. A., A. Barri, M. Hejdysz, and A. Rutkowski. 2016. different doses of coated butyric acid on growth performance and energy utilization in broilers. Poult. Sci. 95:851-859.

Kokoszynski, D., Z. Bernacki, M. Saleh, K. Steczny, and M. Binkowska. 2017. Body conformation and internal organs characteristics of different commercial broiler lines. Braz. J. Poult. Sci. 19:47-52.

Jiménez-Moreno, E., J. M. González-Alvarado, A. González-Serrano, R. Lázaro, and G. G. Mateos. 2009. Effect of dietary fiber and fat on performance and digestive traits of broilers from one to twenty-one days of age. Poult. Sci. 88:2562–2574.

Langhout, D. J., J. B. Schutte, J. Jong, H. Sloetjes, W. A. Verstegen, and S. Tamminga. 2000. Effect of viscosity on digestion of nutrients in conventional and germ-free chicks. Br. J. Nutr. 83:533–540.

Liu, H., J. Wang, T. He, S. Becker, G. Zhang, D. Li, X. Ma. 2018. Butyrate: A double-edged sword for health? Adv. Nutr. 9:21–29.

Mateos, G. G., E. Jiménez-Moreno, M. P. Serrano, and R. P. Lázaro. 2012. Poultry response to high levels of dietary fiber sources varying in physical and chemical characteristics. J. Appl. Poult. Res. 21:156–174.

Nyachoti, C. M., C. F. M. de Lange, B. W. McBride, S. Leeson, and V. M. Gabert. 2000. Endogenous gut nitrogen losses in growing pigs are not caused by increased protein synthesis rates in the small intestine. J. Nutr. 130: 566–72.

Owusu-Asiedu, A., J. F. Patience, B. Laarveld, A. G. Van Kessel, P. H. Simmins, and R. T Zijlstra. 2006. Effects of guar gum and cellulose on digesta passage rate, ileal microbial populations, energy and protein digestibility, and performance of grower pigs. J. Anim. Sci.

84:843–852.

Perera, W. N. U., M. R. Abdollahi, F. Zaefarian, T. J. Wester, G. Ravindran, V. Ravindran. 2019. Influence of inclusion level of barley in wheat-based diets and supplementation of carbohydrase on growth performance, nutrient utilisation and gut morphometry in broiler starters. Br. Poult. Sci. 60:736–748.

Praes, M. F. F. M., A. A. Pereira, S. Sgavioli, K. F. Duarte, J. C. R. Alva, C. H. Domingues, M. M. Puzzoti, and O. M. Junqueira. 2011. Small intestine development of laying hens fed different fiber sources diets and crude protein levels. Rev. Bras. Cien. Avicol. 13:183–188.

Rezaei, M., M. A. Karimi Torshizi, H. Wall, and E. Ivarsson. 2018. Body growth, intestinal morphology and microflora of quail on diets supplemented with micronised wheat fibre." Br. Poult. Sci. 59: 422–29.

SAS Institute. 2020. SAS User's Guide. Statistics. University Edition. SAS Inst. Inc., Cary, NC.

Sacranie, A., B. Svihus, V. Denstadli, B. Moen, P. A. Iji, and M. Choct. 2012. The effect of insoluble fiber and intermittent feeding on gizzard development, gut motility, and performance of broiler chickens. Poult. Sci. 91:693–700.

Sadeghi, A., M. Toghyani, and A. Gheisari. 2015. Effect of various fiber types and choice feeding of fiber on performance, gut development, humoral immunity, and fiber preference in broiler chicks. Poult. Sci. 94:2734–2743.

Shakouri, M. D., P. A. Iji, L. L. Mikkelsen, and A. J. Cowieson. 2009. Intestinal function and gut microflora of broiler chickens as influenced by cereal grains and microbial enzyme supplementation. J. Anim. Physiol. Anim. Nutr. 93:647–658.

Sigleo, S., G. Vahouny. 1984. Effects of dietary fiber constituents morphology and nutrient transport on intestinal. Am. J. Physiol. 246(1 Pt 1):G34-9.

Silva, V. K., V. de Souza Morita, and I. C. Boleli. 2013. Effect of pectin extracted from citrus pulp on digesta characteristics and nutrient digestibility in broilers chickens. Rev. Bras. Zootec. 42:575–583.

Sklan, D., A. Smirnov, and I. Plavnik. 2003. The effect of dietary fibre on the small intestines and apparent digestion in the turkey. Brit. Poult. Sci. 44:735–740.

Stein, H. H., L. L. Berger, J. K. Drackley, G. C. Fahey, Jr., D. C. Hernot, and C. M. Parsons. 2008. Nutritional properties and feeding values of soybeans and their co-products. Pages 613 - 660 in Soybeans, Chemistry, Production, Processing, and Utilization. Johnson, L. A., P. J. White, and R. Galloway, ed. AOCS Press, Urbana IL.

Svihus, B. 2011. The Gizzard: Function, Influence of Diet Structure and Effects on Nutrient Availability. World's Poult. Sci. J. 67: 207–23.

Tellez, G., J. D. Latorre, V. A. Kuttappan, M. H. Kogut, A. Wolfenden, X. Hernandez-Velasco, B. M. Hargis, W. G. Bottje, L. R. Bielke, and O. B. Faulkner. 2014. Utilization of rye as energy source affects bacterial translocation, intestinal viscosity, microbiota composition, and bone mineralization in broiler chickens. Front. Gen. 5: 1–7.

Table 3.1. Ingredient composition of diets fed to male Cobb broilers from 1 to 20 d of age

	CTL	4% SF	6% SF	8% SF	4% SH[1]	6% SH[1]	8% SH[1]
Corn	49.59	49.59	49.59	49.59	52.26	43.79	35.03
Soybean Meal	35.19	35.19	35.19	35.19	32.94	32.38	31.86
Solka floc®	---	2.02	4.04	6.06	---	---	---
Soybean hulls	---	---	---	---	6.12	12.72	19.33
Soybean oil	4.99	4.99	4.99	4.99	3.94	6.68	9.52
Defluorinated Phosphate	1.04	1.04	1.04	1.04	0.26	0.53	0.83
Biofos 16/21P	0.51	0.51	0.51	0.51	1.17	0.98	0.76
Calcium carbonate	0.91	0.91	0.91	0.91	1.24	1.01	0.76
L-Thr	0.12	0.12	0.12	0.12	0.14	0.15	0.17
DL-Met	0.32	0.32	0.32	0.32	0.33	0.35	0.37
Lysine HCl	0.20	0.20	0.20	0.20	0.23	0.22	0.21
Vitamin premix[2]	0.25	0.25	0.25	0.25	0.25	0.25	0.25
Mineral premix[3]	0.15	0.15	0.15	0.15	0.15	0.15	0.15
Sodium Chloride	0.23	0.23	0.23	0.23	0.47	0.29	0.26
Filler (sand)	6.50	4.48	2.46	0.44	0.50	0.50	0.50
Calculated nutrient composition[4]							
Dry matter (%)	90	90	90	90	90	90	90
ME energy (Kcal/kg)	3,000	3,000	3,000	3,000	3,000	3,000	3,000
Protein (%)	21.0	21.0	21.0	21.0	21.0	21.0	21.0
Total Crude Fiber (%)	2.0	4.0	6.0	8.0	4.0	6.0	8.0
Calcium (%)	0.89	0.89	0.89	0.89	0.89	0.89	0.89
Dig. Phosphorus (%)	0.44	0.44	0.44	0.44	0.44	0.44	0.44
Dig. Met (%)	0.63	0.63	0.63	0.63	0.63	0.63	0.63
Dig. TSAA (%)	0.90	0.90	0.90	0.90	0.90	0.91	0.90
Dig. Lys (%)	1.22	1.22	1.22	1.22	1.22	1.22	1.22
Dig. Thr (%)	0.86	0.86	0.86	0.86	0.86	0.86	0.86

[1]Nutrient matrix used for soyhulls contained 871 kcal/kg of ME, 11.2% CP. Av-TSSA: 0.21 Av-lys: 0.59%; Av-Trp: 0.09%; Av-Thr: 0.25; Av-Arg: 0.64, as reported by (Barros-Dourado et al. 2012) .

[2]Vitamin premix provided the following per kilogram of DSM premix: Vit. A, 2,204,586 IU; Vit. D_3, 200,000 ICU; Vit. E, 2,000 IU; Vit. B12, 2 mg; Biotin, 20 mg; Menadione, 200 mg; Thiamine, 400 mg; Riboflavin, 800 mg; d-Pantothenic Acid, 2,000 mg; Vit. B6, 400 mg; Niacin, 8,000 mg; Folic Acid, 100 mg; Choline, 34,720 mg.

[3]Mineral premix includes per kg of premix: Ca, 0.72 g; Mn, 3.04 g; Zn, 2.43 g; Mg, 0.61 g; Fe, 0.59 g; Cu, 22.68 g; I, 22.68 g; Se, 9.07 g.

[4]Values reported as percentages unless noted otherwise.

Table 3.2. Effects of dietary fiber level on the growth performance of male broilers reared to 20 days of age[1]

	Total crude fiber[2]								
Item[3]	CTL	4% SF	6% SF	8% SF	4% SH	6% SH	8% SH	SEM[4]	*P*-value
BWG (g), d7	92	81	86	81	111	95	79	11	0.424
FI (g), d7	122	111	125	121	141	132	131	13	0.771
FCR, d7	1.34[a]	1.47[ab]	1.49[ab]	1.51[ab]	1.27[a]	1.43[ab]	1.73[b]	0.08	0.013
BWG (g), d14	365	342	354	317	399	340	314	21	0.087
FI (g), d14	526	539	553	513	571	562	539	32	0.864
FCR, d14	1.45[a]	1.61[ab]	1.58[ab]	1.61[ab]	1.43[a]	1.65[ab]	1.73[b]	0.06	0.007
BWG (g), d20	756[ab]	729[ab]	739[ab]	625[b]	797[a]	670[ab]	626[b]	37	0.012
FI (g), d20	1347	1396	1418	1404	1409	1325	1413	69	0.943
FCR, d20	1.79[a]	1.94[ab]	1.94[ab]	2.25[b]	1.78[a]	1.98[ab]	2.27[b]	0.10	0.004

[1]Values are the least-square means of 6 replicate pens per treatment with 12 birds per cage. Where applicable, means were separated using Tukey's Honestly Significant Difference Test
[2]Diets were formulated using increasing amounts of solka floc® (SF) or soyhulls (SH) to achieve a total of 4, 6, and 8% crude fiber (4% SF, 6% SF, 8% SF; and 4% SH, 6% SH, 8% SH, respectively), including the fiber from corn and soybean meal.
[3]Mortality-corrected body weight gain (BWG), feed intake (FI), and feed conversion ratio (FCR) per bird.
[4]SEM= largest pooled standard error of the pairwise mean comparison
[a-b]Means within a row not sharing a common superscript differ significantly ($P < 0.05$

Table 3.3. Effects of dietary fiber level on weights and relative organ weights of male broilers reared to 20 days of age[1]

	Total crude fiber[2]								
Item[3]	CTL	4% SF	6% SF	8% SF	4% SH	6% SH	8% SH	SEM[4]	*P*-value
Gizzard, g	24	21	21	21	24	23	22	0.9	0.113
Gizzard, %	2.8^{bc}	2.8^{c}	2.8^{c}	3.0^{abc}	3.0^{abc}	3.4^{a}	3.2^{ab}	0.10	<0.001
Pancreas, g	3	3	3	2	3	3	2	0.16	0.366
Pancreas, %	0.3	0.3	0.3	0.3	0.3	0.4	0.3	0.02	0.702
Liver, g	26^{a}	25^{ab}	25^{ab}	22^{ab}	25^{ab}	20^{b}	20^{b}	1.32	0.001
Liver, %	3.1	3.2	3.3	3.1	3.1	3.0	3.1	0.12	0.183
Duodenum, g	12	12	12	11	12	11	12	0.55	0.244
Duodenum, %	1.5	1.6	1.5	1.6	1.5	1.6	1.7	0.07	0.141
Jejunum, g	30	27	29	26	29	29	31	1.5	0.279
Jejunum, %	3.5^{c}	3.5^{c}	3.7^{bc}	3.7^{bc}	3.6^{bc}	4.2^{ab}	4.5^{a}	0.16	<0.001
Ileum, g	26	24	24	25	28	27	28	1.51	0.214
Ileum, %	3.1^{c}	3.1^{c}	3^{c}	3.5^{bc}	3.4^{bc}	3.9^{ab}	4.0^{a}	0.12	<0.001
Ceca, g	5	6	5	5	7	5	5	0.6	0.136
Ceca, %	0.6	0.7	0.6	0.7	0.9	0.8	0.8	0.07	0.157

[1]Values are the least-square means of 12 replicate birds per treatment. Where applicable, means were separated using Tukey's Honestly Significant Difference Test

[2]Diets were formulated using increasing amounts of solka floc® (SF) or soyhulls (SH) to achieve a total of 4, 6, and 8% crude fiber (4% SF, 6% SF, 8% SF; and 4% SH, 6% SH, 8% SH, respectively), including the fiber from corn and soybean meal.

[3]Duodenum, jejunum, ileum, and ceca were weighed including their contents

[4]SEM= largest pooled standard error of the pairwise mean comparison

[a-b]Means within a row not sharing a common superscript differ significantly ($P < 0.05$

Table 3.4. Villus height, crypt depth, and villi:crypt ratio from the duodenum, jejunum, and ileum of male broilers reared to 20 days of age[1]

		Total crude fiber[2]								
		CTL	4% SF	6% SF	8% SF	4% SH	6% SH	8% SH	SEM[3]	*P*-value
Duodenum	Villi, µm	2194^{ab}	2137^{bc}	2006^{d}	2119^{bcd}	2076^{bcd}	2049^{cd}	2315^{a}	30	<0.01
	Crypt, µm	262	271	259	248	249	269	285	12	0.066
	Ratio, µm	9	8.4	8.3	9	8.8	8.3	8.6	0.3	0.144
Jejunum	Villi, µm	1320^{a}	1181^{bc}	1146^{b}	1160^{bc}	1312^{a}	1117^{c}	1222^{b}	22	<0.01
	Crypt, µm	188^{ab}	181^{ab}	178^{b}	179^{ab}	196^{a}	173^{b}	182^{ab}	5	0.008
	Ratio, µm	7.3	6.7	6.8	6.7	7.1	6.6	7.1	0.2	0.071
Ileum	Villi, µm	641^{bc}	754^{a}	650^{bc}	621^{c}	769^{a}	679^{b}	631^{bc}	15	<0.01
	Crypt, µm	159^{c}	177^{ab}	160^{bc}	146^{c}	180^{a}	146^{c}	162^{bc}	5	<0.01
	Ratio, µm	4.2^{b}	4.5^{ab}	4.3^{b}	4.5^{ab}	4.5^{ab}	5^{a}	4^{b}	0.16	<0.01

[1]Values are the least-square means of 6 replicate birds per treatment. Where applicable, means were separated using Tukey's Honestly Significant Difference Test

[2]Diets were formulated using increasing amounts of solka floc® (SF) or soyhulls (SH) to achieve a total of 4, 6, and 8% crude fiber (4% SF, 6% SF, 8% SF; and 4% SH, 6% SH, 8% SH, respectively), including the fiber from corn and soybean meal.

[3]SEM= largest pooled standard error of the pairwise mean comparison

[a-b]Means within a row not sharing a common superscript differ significantly ($P < 0.05$)

Table 3.5. Effect of dietary fiber level and type on nutrient digestibility of male broilers reared to 20 days of age[1]

	Total crude fiber[2]								
Item	CTL	4% SF	6% SF	8% SF	4% SH	6% SH	8% SH	SEM[3]	*P*-value
DM, %	68.4^{ab}	70.3^{ab}	71.1^{a}	70.0^{ab}	69.2^{b}	62.8^{ab}	62.0^{b}	2.0	0.010
CP, %	82.0	83.7	83.6	82.9	80.7	79.9	80.3	1.3	0.211
AME, Kcal/kg	2,735	2,835	2,892	2,853	2,835	2,632	2,788	79	0.308
Met, %	93.9^{b}	94.3^{b}	93.6^{b}	94.0^{b}	94.6^{b}	94.2^{b}	96.1^{a}	0.3	<0.01
Lys, %	88.9	89.9	88.4	89.3	88.4	89.4	89.4	0.4	0.157
Thr, %	77.6^{bc}	78.7^{bc}	77.1^{c}	77.4^{bc}	79.8^{b}	83.4^{a}	85.5^{a}	0.6	<0.01
Leu, %	84.1^{ab}	84.8^{ab}	83.3^{b}	84.5^{ab}	83.6^{b}	86.0^{a}	86.1^{a}	0.5	<0.01
Tyr, %	82.6^{ab}	83.9^{a}	82.3^{ab}	83.3^{ab}	80.9^{b}	84.0^{a}	83.7^{a}	0.5	<0.01
Phe, %	83.9^{b}	85.1^{ab}	83.4^{b}	84.7^{ab}	83.9^{b}	86.8^{a}	87.1^{a}	0.6	<0.01
Trp, %	90.6	92.3	89.9	90.8	91.3	92.2	92.6	1.0	0.375
Sum EAA[4]	85.8	86.9	85.3	86.1	85.9	88.0	88.3	-	-
Cys, %	70.7^{ab}	73.9^{a}	70.0^{ab}	70.1^{ab}	69.2^{b}	74.2^{a}	72.9^{ab}	1.1	<0.01
Asp, %	80.4^{c}	81.7^{ab}	80.1^{c}	80.7^{bc}	80.0^{c}	84.0^{a}	83.0^{ab}	0.6	<0.01
Ser, %	81.6	83.1	81.2	81.6	81.1	82.9	81.7	0.6	0.175
Glu, %	87.1^{ab}	87.9^{ab}	86.8^{b}	87.5^{ab}	86.7^{b}	88.8^{a}	88.4^{ab}	0.4	<0.01
Pro, %	81.4^{ab}	82.6^{ab}	80.9^{b}	81.5^{ab}	80.5^{b}	83.4^{a}	81.9^{ab}	0.5	<0.01
Gly, %	78.3^{ab}	79.4^{a}	78.3^{ab}	78.1^{ab}	76.1^{b}	78.7^{a}	77.8^{ab}	0.6	<0.01
Ala, %	82.7^{ab}	83.7^{ab}	82.0^{b}	83.0^{ab}	82.0^{b}	84.7^{a}	84.4^{ab}	0.6	<0.01
Sum NEAA[4]	80.6	82.0	80.2	80.7	79.6	82.6	81.7	-	-
Ratio EAA/NEAA[5]	1.06	1.06	1.06	1.07	1.08	1.07	1.08	-	-

[1]Values are the least-square means of 6 replicate pens per treatment. Where applicable, means were separated using Tukey's Honestly Significant Difference Test

[2]Diets were formulated using increasing amounts of solka floc® (SF) or soyhulls (SH) to achieve a total of 4, 6, and 8% crude fiber (4% SF, 6% SF, 8% SF; and 4% SH, 6% SH, 8% SH, respectively), including the fiber from corn and soybean meal.

[3]SEM= largest pooled standard error of the pairwise mean comparison

[4]Sum of essential amino acids (EAA) and non-essential amino acids (NEAA) was calculated as the sum of the percentage digestibility of all the amino acids in each group divided by the number of amino acids in each group.

[5]The ration of EAA: NEAA was calculated by dividing the sum EAA by NEAA.

[a-b]Means within a row not sharing a common superscript differ significantly ($P < 0.05$)

CHAPTER 4

THE USE OF SEMI-PURIFIED DIETS TO EVALUATE THE EFFECT OF SOLUBLE TO INSOLUBLE FIBER RATIOS ON THE GROWTH PERFORMANCE, ORGAN GROWTH, GUT MORPHOLOGY, NUTRIENT DIGESTIBILITY, AND INTESTINAL VISCOSITY OF BROILER CHICKENS[1]

[1]Tejeda, O. J. and W. K. Kim. Submitted to Poultry Science.

Abstract

An experiment was conducted to determine the effects of soluble to insoluble fiber ratios on the growth performance, organ growth, intestinal morphology, intestinal viscosity and nutrient digestibility. A total of 432 one-day old Cobb® male broilers were randomly assigned to six dietary treatments and reared to 21 days of age in battery cages (n=6 replicates per treatment). A semi-purified basal diet with 0% crude fiber was formulated to which different soluble to insoluble fiber ratio was added. All birds were fed a common corn-soybean meal diet during the first 7 days to ensure proper organ growth development, before being exposed to the semi-purified experimental diets. Growth performance was measured on days 14 and 21. Ileal samples were collected on days 14 and 21 for analyses of intestinal viscosity. On day 21, ileal digesta was collected from 7 birds per replicate for nutrient digestibility analyses, and one bird was used to collect sections of the duodenum, jejunum, and ileum for intestinal morphology analyses. Higher soluble to insoluble fiber ratios resulted in poorer gain per bird and feed efficiency from d 7 to 21 ($P < 0.01$). No Statistical differences were observed in the organ growth among the dietary treatments ($P > 0.05$). No differences in the duodenal villus to crypt ratio were observed among the dietary treatments ($P > 0.05$). However, the group fed the lowest soluble to insoluble fiber ratio had the smallest jejunal villus to crypt ratio, and the biggest ileal villus to crypt ratio ($P < 0.001$). Decreases in digestibility of dry matter, apparent metabolizable energy and crude protein were observed in increases in the soluble to insoluble fiber ratio ($P < 0.05$). No statistical differences in intestinal viscosity were observed from d 7 to 14 ($P > 0.05$). However, higher soluble to insoluble fiber ratios showed a tendency of higher intestinal viscosity on day 21 ($P = 0.09$). In conclusion, high soluble to insoluble fiber ratios reduce growth performance and nutrient digestibility.

Key words: broiler chicken, dietary fiber, digestibility, gut morphology, organ growth

Introduction

Dietary fiber (DF) consists of a group of heterogenous and complex carbohydrates that form part of the plant cell wall found in all plant tissues including feedstuffs commonly used in broiler diets (Keegstra, 2010; Knudsen, 2014). Such carbohydrates are organized in a tightly-associated three-dimensional structure containing fibrillar polysaccharides (i.e. insoluble fibers) and matrix polysaccharides (i.e. soluble fibers) (Hetland et al., 2004; Keegstra, 2010). The ratios of these components vary among feedstuffs, plant varieties, stage of maturity, and environmental conditions (Jaworski et al., 2015; Knudsen, 2014; Nguyen et al., 2019). Cereal grains such as wheat, barley and rye contain soluble to insoluble fiber ratios of 2:9, 5:12, and 5:9, respectively and have been associated with negative modulation of nutrient digestibility (Friesen et al., 1992; Perera et al., 2019), intestinal microbiota (Józefiak et al., 2010), and growth performance (Jacob et al., 2012; McNab and Smithard, 1992). Soluble to insoluble fiber ratios found in corn is as low as 0.54:9 (Nguyen et al., 2019) causing no antinutritional problems when fed to poultry.

The matrix polysaccharide, formed by viscous and non-viscous soluble fibers, have the ability to reduce the passage rate of the digesta in the small intestine resulting in lower enzyme diffusion, substrate hydrolysis, diffusion of nutrients and subsequent impairment of nutrient digestibility and performance (Jiménez-Moreno et al., 2009; Silva et al., 2013). All these changes in nutrient digestibility has been shown to have a profound effect in the modulation of intestinal microbiota by increasing the substrates in the intestinal lumen (Kiarie, Romero and Nyachoti, 2013; Tellez et al., 2014). On the other hand, insoluble fibers increase the bulk of the digesta which results in a faster passage rate in the small intestine (Hetland and Svihus, 2001). Insoluble

fibers are hydrolyzed and utilized to a lesser extent compared to the soluble portion, making them less influential in the modulation of intestinal microbiota (Hetland et al., 2004; Langhout, 1998). The opposite roles played by both fiber types indicate the potential to cause changes in nutrient utilization, intestinal modulation, and growth performance when using different fiber ratios. However, there is scarce information regarding how different soluble to insoluble fiber ratios can affect the nutrition of poultry species. Therefore, the objective of this study was to evaluate the effects of different soluble to insoluble fiber ratios on the growth performance, nutrient digestibility, gut morphology, intestinal viscosity, and organ growth in broiler chickens.

Materials and methods

General procedures

The experiment was approved by the Institutional Animal Care and Use Committee (IACUC) of the University of Georgia (Athens, Georgia, United States). A stock of 640 one-day-old Cobb500™ broiler chicks were provided with a standard corn-soybean meal diet from 1 to 6 d of age. At day 7 of age, all broiler chickens from the stock group were weighed, and a total of 432 chicks were reallocated in a completely randomized design with six dietary treatments and six replicates of 12 birds each, such that the average pen body weight was similar. The soluble (pectin) to insoluble (cellulose) fiber ratio was the main factor. All cages were equipped with one drinker and one feeder, providing *ad-libitum* access to water and semi-purified mash feed until 21 d of age. Temperature and lighting program followed the recommendation of Cobb Broiler Management Guide (Cobb-vantress, 2018).

Dietary Treatments

A standard corn and soybean meal-based diet formulated to meet the nutrients requirements specified by Cobb500 performance and nutritional guide (Cobb-Vantress, 2018)

was fed to all birds during the first week of the grow-out period (**Table 4.1**). Experimental diets were fed from 7 to 21 d of age. To ensure that experimental diets were fiber-free, a basal diet was formulated to contain 0% crude fiber (**Table 4.2**), to which purified cellulose (**SF**: 99% cellulose, Solka floc®, Skidmore, Schollcraft, MI) and fruit pectin (**FP**: dextrose, pectin, fumaric acid, Cranford, NJ) were added at various experimental ratios (**Table 4.3**) as a source of insoluble (**IF**) and soluble fiber (**SF**), respectively, by replacing an inert filler (sand). All diets, except the control, had a total of 9% added fiber provided by the different soluble to insoluble fiber ratios (**SF:IF**). The control diet was mixed by adding Solka floc to the basal diet to achieve 2% crude fiber. All dietary treatments were formulated to meet or exceed the nutrient requirements specified by Cobb500 performance and nutritional guide (Cobb-Vantress, 2018) and were provided as mash. For ileal nutrient digestibility analyses, chromic oxide (Cr_2O_3, Sigma Aldrich, St. Louis, MO) was added at 0.3% as an indigestible marker to all diets from 14-21 d.

Growth Performance and Organ Weights

The birds and feed were weighed on 14 and 21 d of age to determine mortality-corrected body weight gain (**BWG**), mortality-corrected feed intake (**FI**), and mortality-corrected feed conversion ratio (**FCR**). Mortality was recorded twice daily. On d 21, one average bird per cage was euthanized, and empty gizzard, liver, small intestine, and ceca were weighed to determine the relative organ weight.

Intestinal histomorphology

On d 21, samples from the duodenum, jejunum and ileum (~ 2cm long) were collected from one average bird per replicate cage (n = 6 per treatment). Intestinal samples were collected and stored in 10% neutral-buffered formalin and left in solution for a minimum period of 48

hours for tissue fixation. During slide preparation, the tissues were dehydrated in increasing amounts of ethanol, diaphanized in dimethylbenzene, and fixed in paraffin. Subsequently, tissue sections with a thickness of 4-µm on slides were stained using Hematoxylin and Eosin (H&E) procedures. Pictures were taken using a light microscope (10x eyepiece and 1.6x magnification; Leica DC500 camera, Leica Mycrosystems Inc., Buffalo Groove, IL). Measurements for villi height and crypt depth were taken using ImageJ software (Image Processing and Analysis in JAVA – ImageJ 1.52r, National Instituted of Health).

Nutrient digestibility

On d 21, seven birds per cage were euthanized, and ileal digesta was collected from two-thirds of the distal ileum (about 1 inch anterior to ileocecal junction). The digesta samples were pooled and dried for analyses of dry matter, apparent metabolizable energy (**AME**) and crude protein. The chromium concentration was measured in duplicate according to Dansky and Hill (1952). The gross energy of experimental diets and dried ileal digesta were evaluated using a bomb calorimeter (IKA Calorimeter C1, IKA Works Inc., Wilmington, NC). The crude protein (N × 6.25) was analyzed using a LECO nitrogen analyzer (LECO, St. Joseph, MI, USA). The apparent ileal digestibility (AID) of dry matter, apparent metabolizable energy (AME), and crude protein were calculated using the following equation:

$$AID, \% = 100\left[1 - \left(\frac{Cr_{feed}}{Cr_{dig}}\right) \times \left(\frac{Nutrient_{dig}}{Nutrient_{feed}}\right)\right]$$

where Cr_{feed} and Cr_{dig} are the chromium dioxide in feed and ileal digesta, respectively; and $nutrient_{dig}$ and $nutrient_{feed}$ are the nutrient in ileal digesta and feed, respectively.

Intestinal viscosity

On days 14 and 21 of age, one bird per cage was euthanized, and intestinal digesta were collected from the Meckel's diverticulum to the ileocolonic junction. Fresh digesta were

centrifuged at 12,600 × g for 5 minutes, and the supernatants were collected for viscosity measurements using a cone and plate Brookfield DV-II + Programmable viscometer (Brookfield engineering laboratories, Inc, Middleboro, MA, USA). A water bath control connected to the cone was used to keep the temperature of the samples at 40 ºC. The viscosity was measured at 10 rpm using a CPE-40 spindle.

Statistical analyses

Soluble to insoluble fiber ratio level was used as the fixed effect in the model. Pen was used as the experimental unit for growth performance and nutrient digestibility; bird was used as the experimental unit for organ growth, intestinal morphology, and intestinal viscosity. Data were analyzed using one-way Analysis of Variance by the following model:

$$Y_{ij} = \mu + \alpha_i + \varepsilon_{ij}$$

where Y_{ij} represents the value for each random variable; μ is the overall mean; α_i are the fixed factor level effects corresponding to the ith treatment such that $\Sigma\alpha_i=0$; and the random errors ε_{ij} are identically and independently normally distributed with a mean 0 and a variance σ. All statistical procedures were performed using SAS University Edition (SAS Institute, 2021). In case of significant differences, means were separated using the Tukey's honesty significant difference (HSD) option. For all hypothesis tests, statistical significance was considered at $P < 0.05$.

Results

Growth performance and organ weights

The results for growth performance are shown in Table 4.4. No statistical differences were observed on the growth performance on day 14 ($P > 0.05$). However, on day 21, bird weight gain was higher in birds fed the lowest SF:IF ratio (1:4), showing an increase in body

weight with decreases in soluble to insoluble fiber ratios; furthermore, the group fed the highest SF:IF ratio had the poorest weight gain among treatments ($P < 0.001$). The worst and the best feed conversion ratio were observed in the group with the highest and the lowest SF:IF ratio, respectively, among the dietary treatments ($P < 0.01$). No differences in feed intake were observed during d 7 to 21 ($P = 0.399$). Results for organ growth are shown in Table 4.5. No significant differences in the organ growth were observed among dietary treatments on day 14 or 21 ($P > 0.05$).

Intestinal histomorphology

Results for intestinal histomorphology are shown in Table 4.6. The group fed 1:2 (SF:IF) ratio had shorter duodenal villus height and crypt depth compared to the control, 4:1 or 1:4 ratio groups ($P < 0.006$). The 1:2 ratio group had the shortest duodenal villus compared to the groups fed 4:1, 1:1, and 1:4 ($P = 0.006$). The duodenal crypt depth was the lowest in the groups fed 1:2 ratios among dietary treatments ($P = 0.023$). No significant differences were observed in the duodenal villus to crypt ratio among dietary treatments ($P > 0.05$). The treatment fed the lowest SF:IF ratio (1:4) had the shortest jejunal villus and jejunal villus to crypt ratio compared to the rest of dietary treatments ($P < 0.001$). No significant differences were observed in jejunal crypt depth among dietary treatments ($P > 0.05$). The jejunal villus to crypt ratio was worsen by the highest inclusion of IF ($P < 0.01$). Ileal villus height was the lowest in the group fed the highest SF:IF ratio among dietary treatments ($P < 0.001$). The ileal crypt depth was higher in the group with 1:1 ratio among dietary treatments ($P < 0.001$). The ileal villus to crypt ratio was lower in the groups 4:1, 2:1, and 1:1 compared to the group fed 1:4 ratio ($P < 0.001$).

Nutrient digestibility and Intestinal viscosity

The results for nutrient digestibility and intestinal viscosity are shown in Table 4.7. Inclusion of insoluble fiber in groups 1:1 and 1:4 increased the digestibility of dry matter by 5% (85.77%) compared to the control group (81.97%) and the 4:1 group (81.71%) containing highest levels of soluble fiber ($P = 0.012$). The group fed the lowest SF:IF ratio had an improvement in the digestibility of apparent metabolizable energy of 150 kcal compared to the rest of dietary treatments, except the 1:1 ratio group ($P < 0.01$). The digestibility of crude protein was reduced by 21% in the group 4:1 with the highest SF:IF ratio when compared to the control group and the group fed 1:4 ratio ($P = 0.005$). Numerical but not statistically significant decreases in intestinal viscosity were observed with reductions of SF:IF ratios ($P > 0.05$) on day 14. However, on day 21, there was a significant decrease in intestinal viscosity with decreases in SF:IF ratios where the control group had the lowest intestinal viscosity values (3.78 mPas), and treatment 2, with highest SF:IF ratio (4:1), had the highest viscosity values (11.6 mPas) ($P = 0.009$). Birds fed treatment 6 did not differ in intestinal viscosity compared to those fed the control group.

Discussion

Growth performance and intestinal and organ weights

To adequately assess the sole impact of soluble and insoluble fiber, different soluble to insoluble fiber ratios were given to broilers in the form of semi-purified diets containing 0% crude fiber, yet, formulated to have the same nutrient amounts. Despite that all diets were formulated to contain the same amount of nutrients, there were differences in growth performance among the dietary treatments; it can be attributed to their respective fiber ratios. Birds fed the highest SF:IF fiber ratio had the lowest weight gain among the dietary treatments. Replacements of pectin by cellulose ameliorated the negative impact of pectin restoring the

weight gain and resulting in a gain similar to the control group in the current study. In accordance with these results, Langhout (1998) reported a depressed performance of chicks fed highly methylated pectin. The negative impact of soluble fibers has been extensively reported by other researchers (Langhout, 1998; Sadeghi, Toghyani et al., 2015; White et al., 1981). Such negative effects are generally attributed to the ability of soluble fibers to interact with water molecules and create viscous solution in the gastrointestinal tract of the chicken (Chaplin, 2003), promoting changes in nutrient dynamics and impairment in performance. In the present experiment, the weight gain and the FCR differed by 27% and 19%, respectively, between the highest and the lowest SF:IF ratio. In other words, birds from the group fed with the highest SF:IF ratio was about one third lighter and needed about one fifth more feed to compensate for the reduction in growth caused by SF. In the present experiment, insoluble fibers resulted to be more inert when given in amounts as high as 7.2% in the diet when compared to soluble fibers. Contrary to the results from the present experiment, other researchers have reported that IF levels higher than 5% can result in reduction in performance parameters (Cao et al., 2003; Sklan, Smirnov et al., 2003). Nevertheless, it is important to bear in mind that all the diets used in the present experiment, including the control group, were semi-purified diets using purified sources of fibers (i.e. pectin and cellulose). Therefore, the natural composition of DF and the chemical arrangement within the matrix of polysaccharides in the cell wall of common feedstuffs play an important role determining the functional role of the dietary portion in the diet. This is because the cell walls of vegetable feedstuffs have a combination of different pectic polysaccharides (i.e. soluble fibers) and hemicellulosic polysaccharides (i.e. insoluble fibers) (Keegstra, 2010), which confers different functional roles based on their composition and combinations and can vary even among the same feed ingredients as reported by other researchers (Jaworski et al., 2015;

Knudsen, 2014). Nevertheless, it has been clearly demonstrated in the present experiment that higher inclusions of IF are less counterproductive to performance parameters compared to SF.

The lack of significant differences in digestive organ growth observed in the present experiment was not expected. Several other researchers have reported the role of dietary fiber on the modulation of the weights of digestive organs including the gizzard and proventriculus (Amerah et al., 2009; González-Alvarado et al., 2007), small intestines (Sacranie et al., 2012), liver (Langhout and Schutte, 1996; Rezaei, Karimi et al., 2018), and ceca (Jiménez-Moreno et al., 2009). In fact, Saki et al. (2011) conducted a similar experiment feeding different pectin to cellulose ratios (2:1, 1.5:1:5, and 1:2) and found that the ratio 2:1 pectin to cellulose resulted in an increased small intestine weight at day 42. However, these authors used corn grain as the main source of energy including a higher level of dietary fiber than the one coming from the purified sources. A clear explanation for these differences is because natural fibers found in commonly-used feedstuffs are known for their high degree of polymerization conferring them the ability to exert a mechanical stress upon digestive organs, resulting in heavier organ weights (Harholt et al., 2010; Keegstra, 2010). Thus, this contrast in results could be attributed to the changes in the degree of polymerization observed in purified sources of dietary fiber (Hivechi and Bahrami, 2016) that may reduce the ability of such fibers to modulate organ growth. Furthermore, particle size of dietary fiber is another important parameter determining the overall functionality of dietary fiber in terms of organ growth (Amerah et al., 2007). Therefore, feeding natural fibers contained in vegetable feedstuffs results in either the inclusion of unseen chemical components presents in the plant cell walls or the synergistic interaction of fiber components by themselves that exert functional roles in the modulation of digestive organs.

Intestinal morphology

In poultry species, the presence of dietary fiber can increase reverse peristalsis happening in the upper portion of the gastrointestinal tract (i.e. gastroduodenal reflux) which mainly includes the proventriculus and the duodenum, with potential to modify intestinal morphology (Sacranie et al., 2012). In the present study, duodenal villus height and crypt depth were higher in the groups fed higher SF:IF ratios. Similar to these results, (Chiou et al., 1996) reported an increase in duodenal villus when geese were fed pectin and shortening of duodenal villus when fed lignin. On the other hand, (Viveros et al., 1994) reported shortening, thickening, and atrophy of the jejunal villi in birds fed diets containing soluble fiber in the form of β-glucans when compared to the control group. Furthermore, it has been suggested that soluble fibers increase the intestinal villus atrophy by increasing intestinal viscosity and increasing the rate of epithelial cell losses (Jha et al., 2019). However, Tejeda and Kim (2020) reported that broilers fed 8% CF, using soyhulls containing approximately 30% pectin as source of fiber, increased the duodenal villus height compared to groups fed 4, 6 and 8% CF using cellulose. Praes et al. (2011) also reported that laying hens fed 7.5% soyhulls had an improvement in duodenal and jejunal villi.

The treatment fed the lowest SF to IF ratio (1:4) had the shortest jejunal villus height and jejunal villus to crypt ratio compared to the rest of dietary treatments. In contrast to these results, other researchers have reported an increased villus height in poultry species fed high levels of insoluble fibers (Chiou et al., 1996; Sklan et al., 2003). Therefore, the lack of stimulus (i.e. abrasivity) exerted by purified cellulose when compared to other sources of fiber might be the main reason behind the shortening of intestinal villi. Dahlke et al. (2003) reported the increase in duodenal villus with increase in corn particle size. In the present study, the increase in duodenal and jejunal villi can be attributed to the ability of pectins to reduce passage rate and increase the

physical stimulus on intestinal villus when compared to cellulose, and the fact that both purified sources of fiber were given in small particle size, may result in under-stimulation of epithelial cell within the small intestines. Therefore, it is of importance to account for the particle size of the fiber, besides the type and inclusion levels. In the present experiment, no significant differences were observed in jejunal crypt among the dietary treatments. However, inclusion of high SF reduced ileal villus, crypt and their respective ratio. These results are in accordance with Sadeghi et al. (2015) who reported that broilers fed soluble fibers from beet pulp had lower ileal villus compared to the control group. The differences in intestinal morphology parameters in the different sections of the small intestine are associated to the different functions of each section. The duodenum serves as the main site where digesta coming from the proventriculus mixes with pancreatic enzymes and functions as a bridge during the process of reverse peristalsis, allowing the movement of digesta from the small intestine to the proventriculus (Duke, 1982). These refluxes can modulate intestinal morphology due to the abrasion associated with the movements of digesta and it has been reported that such refluxes are improved with the presence of natural insoluble fibers (Sacranie et al., 2012) where the presence of purified sources of fiber with very small particle size such in the case of the present experiment reduces physical stimulus reducing villus height. In the case of the jejunum, its villus height and crypt depth is generally modulated by insoluble fibers with bigger particles sizes (Mateos et al., 2012). In the present experiment the mean particle size of the purified cellulose used as insoluble fiber was 100µm which is associated to the under-stimulation of jejunal villus and the respective reduction in villus height in the duodenum and jejunum. The fact that the ileal villus and villus to crypt ratio was lower in the groups fed the highest SF:IF ratios can be associated to the development of pathogenic intestinal bacteria due to the high intestinal viscosity in groups fed such diets. This is supported

by Wagner and Thomas, (1978) who reported that ileum anaerobe counts of chickens fed diets containing rye or pectin were 2 or 3 cycles higher than chickens fed corn and soybean meal diets. Viveros et al., (1994), also reported shorter and thicker villi in birds fed soluble fibers from barley-based diets.

Measurements of villus have been used as a criterion for the likely estimation of absorptive capacity of the small intestine, and crypt as an indicator of the potential level of epithelial cell turnover (Montagne et al., 2003). However, changes in intestinal histomorphological parameters must be accompanied by data in nutrient digestibility and general growth performance to determine their impact on production. This is because changes in intestinal morphology are generally associated to changes in nutrient dynamics where lower nutrient absorption can increase villus as a means to offset the reduction in nutrient intake at the level of the brush border. This has been reported when feeding highly fiber-diluted diets fed to broilers (Sadeghi et al., 2015; Sklan et al., 2003). In the case of the present experiment, the increase in jejunal villus height could be attributed to the need to increase nutrient absorption to compensate for growth, whereas the increase in jejunal to crypt ratio could be associated to the lack of abrasion that results in no change in crypt depth leading to increase of the final villus to crypt ratio.

Nutrient digestibility and viscosity

The apparent digestibility of dry matter was improved with additions of cellulose. The group fed the highest SF:IF ratio (4:1) had similar dry matter digestibility compared to the control group. Similarly, Tejeda and Kim (2020) observed an increase in dry matter digestibility in broiler feed cellulose compared to the group fed soybean hulls at 8% crude fiber with 30% pectin. Silva et al. (2013) also reported a reduction in apparent digestibility of dry matter in

broilers fed 10 to 50 g/ kg. In the present experiment, lower ratios of SF:IF increased the digestibility of energy and crude protein compared to higher ratios. The differences in dry matter, and energy digestibility between the group fed SF:IF ratios of 4:1 and 1:4 were 5%, whereas the difference in crude protein digestibility was 21%, with the lowest SF:IF ratio group (higher cellulose) having higher digestibility compared to the highest group (4:1) with higher pectin inclusion. In accordance to these results, Langhout (1998) reported that feeding pectin to broilers resulted in reduction in nitrogen retention, energy and starch digestibility. This reduction in nutrient digestibility can be attributed to the increase in intestinal viscosity. The increases in the SF to IF ratios resulted in increased intestinal viscosity which is correlated with reduction in nutrient digestibility on day 21. This reduction in nutrient digestibility is associated to reduction in enzymatic diffusion due to the accumulation of viscous materials along the mucosal surface, reducing the nutrient break down and nutrient absorption (Hetland et al., 2004; Jiménez-Moreno et al., 2009). Furthermore, the presence of viscous dietary components increases the thickness of the unstirred water of the mucosa layer by interacting with the glycocalyx of the intestinal brush border, increasing the production of mucus and leading to a decrease in the dispersion of nutrients and subsequent reduction in nutrient digestibility (Pluske et al., 2009). This is the reason why the soluble portion of the dietary fibers is regarded to most determinant factor affecting the nutritive value in poultry diets. Other soluble fibers such as guar gum and wheat pentosans have been reported to decrease digestibility of lipids, crude protein, and starches, resulting in decreases in growth performance (Choct et al., 2010).

Conclusions

Different soluble to insoluble fiber ratios are important factors determining the overall functionality of dietary fiber in terms of growth performance and nutrient digestibility. In the

present study, higher inclusions of purified pectin drastically increased intestinal viscosity and reduced nutrient digestibility, resulting in a depressed growth. Intestinal histomorphological parameters seemed not to be matched with the prediction of nutrient digestibility as they have been regarded by other researchers. Therefore, changes in intestinal morphology must be associated with other more stable parameters (i.e. growth performance, nutrient digestibility) to guarantee the accuracy in the determination of the functionality of dietary fiber. In summary, insoluble fibers can be regarded as inert dietary components when added to diets isocaloric and isonitrogenous, and their addition to diets can help reduce the negative impacts of soluble fibers. Further research evaluating the impact of fiber particle size and inclusion level is encouraged to better understand the physiological and nutritional role of dietary fiber.

References

Amerah, A. M., V. Ravindran, and R. G. Lentle. 2009. Influence of insoluble fibre and whole wheat inclusion on the performance, digestive tract development and ileal microbiota profile of broiler chickens. Br. Poult. Sci. 50:366–375.

Amerah, A. M., V. Ravindran, R. G. Lentle, and D. G. Thomas. 2007. Feed particle size: Implications on the digestion and performance of poultry. W. Poult. Sci. J. 63:439–455.

Cao, B. H., X. P. Zhang, Y. M. Guo, Y. Karasawa, and T. Kumao. 2003. Effects of dietary cellulose levels on growth, nitrogen utilization, retention time of diets in digestive tract and caecal microflora of chickens. Asian-Austr. J. Anim. Sci. 16:863–866.

Chaplin, M. F. 2003. Fibre and water binding. Proc. Nutr. Soc. 62:223–227.

Chiou, P. W. S., T. W. Lu, J. C. Hsu, and B. Yu. 1996. Effect of different sources of fiber on the intestinal morphology of domestic geese. Asian-Austr. J. Anim. Sci. 9:539–550.

Choct, M., and G. Annison. 1992. The inhibition of nutrient digestion by wheat pentosans. Br. J. Nutr. 67:123–132.

Cobb-vantress. (2018). Broiler Management Guide. Accessed Jan. 2021. https://www.cobb-vantress.com/products/cobb500.

Cobb-Vantress. (2018). Broiler Performance and Nutrition Supplement Cobb500. Accessed Jan. 2021. http://www.cobb-vantress.com/docs/default-source/cobb-500-guides/cobb500-broiler-performance-nutrition-supplement-(english).pdf

Dahlke, F., A. Ribeiro, A. Kessler, A. Lima, and A. Maiorka. 2003. Effects of corn particle size and physical form of the diet on the gastrointestinal structures of broiler chickens. Rev. Bras. Ciên. Av. 5:61–67.

Duke, G. E. 1982. Gastrointestinal motility and its regulation. Poult. Sci. 61:1245–1256.

Friesen, O. D., W. Guenter, R. R. Marquardt, and B. A. Rotter. 1992. The effect of enzyme supplementation on the apparent metabolizable energy and nutrient digestibilities of wheat, barley, oats, and rye for the young broiler chick. Poult. Sci. 71:1710–1721.

González-Alvarado, J. M., E. Jiménez-Moreno, R. Lázaro, G. Mateos. 2007. Effect of type of cereal, heat processing of the cereal, and inclusion of fiber in the diet on productive performance and digestive traits of broilers. Poult. Sci. 86:1705–1715.

Harholt, J., A. Suttangkakul, and H. V. Scheller. 2010. Biosynthesis of pectin. Plant Physiol. 153:384–395.

Hetland, H., M. Choct, and B. Svihus. 2004. Role of insoluble non-starch polysaccharides in poultry nutrition. W. Poult. Sci. J. 60:415–422.

Hetland, H., and B. Svihus. 2001. Effect of oat hulls on performance, gut capacity and feed passage time in broiler chickens. Br. Poult. Sci. 42:633–637.

Hivechi, A., and S. H. Bahrami. 2016. A new cellulose purification approach for higher degree of polymerization: Modeling, optimization and characterization. Carb. Polym. 152:280–286.

Jacob, J. P., A. J. Pescatore. 2012. Using barley in poultry diets-a review. J. Appl. Poult. Res. 21: 915–940.

Jaworski, N. W., H. N. Lærke, K. E. Bach Knudsen, and H. H. Stein. 2015. Carbohydrate composition and in vitro digestibility of dry matter and nonstarch polysaccharides in corn, sorghum, and wheat and coproducts from these grains1. J. Anim. Sci. 93:1103–1113.

Jha, R., J. M. Fouhse, U. P. Tiwari, L. Li, and B. P Willing. 2019. Dietary fiber and intestinal health of monogastric animals. Front. Vet. Sci. 6:1–12.

Jiménez-Moreno, E., J. M. González-Alvarado, A. González-Serrano, R. Lázaro, and G. Mateos. 2009. Effect of dietary fiber and fat on performance and digestive traits of broilers from one to twenty-one days of age. Poult. Sci. 88:2562–2574.

Józefiak, D., A. Rutkowski, S. Kaczmarek, B. B. Jensen, R. M. Engberg, O. HØjberg. 2010. Effect of β-glucanase and xylanase supplementation of barley- and rye-based diets on caecal microbiota of broiler chickens. Br. Poult. Sci. 51:546–557.

Keegstra, K. 2010. Plant cell walls. Plant Physiol. 154:483–486.

Kiarie, E., L. F. Romero, and C. M. Nyachoti. 2013. The role of added feed enzymes in promoting gut health in swine and poultry. Nutr. Res. Rev. 26:71–88.

Knudsen, K. E. B. 2014. Fiber and nonstarch polysaccharide content and variation in common crops used in broiler diets. Poult. Sci., 93:2380–2393.

Langhout, D. J. 1998. The role of the intestinal flora as affected by non-starch polysaccharides in broiler chicks. PhD thesis, Wageningen University Research.

Langhout, D. J., and J. B. Schutte. 1996. Nutritional Implications of Pectins in Chicks in Relation to Esterification and Origin of Pectins. Poult. Sci. 75:1236–1242.

Mateos, G., E. Jimenez-Moreno, M. P. Serrano, and R. P. Lazarp. 2012. Poultry response to high levels of dietary fiber sources varying in physical and chemical characteristics. J. Appl. Poult. Res. 21:156-174.

Maisonnier, S., J. Gomez, B. Carré. 2010. Nutrient digestibility and intestinal viscosities in broiler chickens fed on wheat diets , as compared to maize diets with added guar gum. Br. Poult. Sci. 42:102-110.

McNab, J. M., and R. R. Smithard. 1992. Barley β-Glucan: an antinutritional factor in poultry feeding. Nutr. Res. Rev. 5:45-60.

Montagne, L., J. R. Pluske, D. J. Hampson. 2003. A review of interactions between dietary fibre and the intestinal mucosa, and their consequences on digestive health in young non-ruminant animals. Anim. F. Sci. Technol. 108:95–117.

Nguyen, N., M. Jacobs, J. Li, C. Huang, D. Li, D. M. Navarro, H. H. Stein, and N. W. Jaworski, N. W. 2019. Technical note: Concentrations of soluble, insoluble, and total dietary fiber in feed ingredients determined using method AOAC 991.43 are not different from values determined using method AOAC 2011.43 with the AnkomTDF dietary fiber analyzer. J. of Anim. Sci. 97:3972–3983.

Perera, W. N. U., M. R. Abdollahi, F. Zaefarian, T. J. Wester, G. Ravindran, V. Ravindran. 2019. Influence of inclusion level of barley in wheat-based diets and supplementation of carbohydrase on growth performance, nutrient utilisation and gut morphometry in broiler starters. Br. Poult. Sci. 60:736–748.

Pluske, J. R., J. C. Kim, D. E. McDonald, D. W. Pethick, and D. J. Hampson. 2009. The Weaner

Pig: Nutrition and Management. Pages 81-112 in non-starch polysaccharides in the diets of young weaned piglets. Ed. M. A. Varley, and J. Wiseman.

Praes, M. F. F. M., A. A. Pereira, S. Sgavioli, K. F. Duarte, J. C. Alva, C. H. Domingues, M. M. Puzzoti, and O. M. Junqueira. 2011. Small intestine development of laying hens fed different fiber sources diets and crude protein levels. Rev. Bras. Cie. Av. 13:183–188.

Rezaei, M., M. A. Karimi Torshizi, H. Wall, and E. Ivarsson. 2018. Body growth, intestinal morphology and microflora of quail on diets supplemented with micronised wheat fibre. Br. Poult. Sci. 59:422–429.

Sacranie, A., B. Svihus, V. Denstadli, B. Moen, P. A. Iji, and M. Choct. 2012. The effect of insoluble fiber and intermittent feeding on gizzard development, gut motility, and performance of broiler chickens. Poult. Sci. 91:693–700.

Sadeghi, A., M. Toghyani, A. Gheisari. 2015. Effect of various fiber types and choice feeding of fiber on performance, gut development, humoral immunity, and fiber preference in broiler chicks. Poult. Sci. 94:2734–2743.

Saki, A. A., H. R. Matin, P. Zamani, M. M. Tabatabai, M. Vatanchian. 2011. Various ratios of pectin to cellulose affect intestinal morphology, DNA quantitation, and performance of broiler chickens. Liv. Sci. 139:237–244.

Silva, V. K., V. Souza Morita, I. C. Boleli. 2013. Effect of pectin extracted from citrus pulp on digesta characteristics and nutrient digestibility in broilers chickens. Rev. Bras. Zootec. 42:575–583.

Sklan, D., A. Smirnov, I. Plavnik. 2003. The effect of dietary fibre on the small intestines and apparent digestion in the turkey. Br. Poult. Sci. 44:735–740.

Tejeda, O. J., and W. K. Kim. 2020. The effects of cellulose and soybean hulls as sources of

dietary fiber on the growth performance, organ growth, gut histomorphology, and nutrient digestibility of broiler chickens. Poult. Sci. 99:6828–6836.

Tellez, G., J. D. Latorre, V. A. Kuttappan, M. H. Kogut, A. Wolfenden, X. Hernandez-Velasco, B. Hargis, W. G. Bottje, L. R. Bielke, O. B. Faulkner. 2014. Utilization of rye as energy source affects bacterial translocation, intestinal viscosity, microbiota composition, and bone mineralization in broiler chickens. Front. Gen. 5:1–7.

Viveros, A., A. Brenes, M. Pizarro, M. Castano. 1994. Effect of enzyme supplementation of a diet based on barley, and autoclave treatment, on apparent digestibility, growth performance and gut morphology of broilers. Anim. F. Sci. Technol. 48:237–251.

Wagner, D. D., O. P. Thomas. 1978. Influence of diets containing rye or pectin on the intestinal flora of chicks. Poult. Sci. 57:971-975.

White, W. B., H. R. Bird, M. L. Sunde, N. Prentice, W. C. Burger, and J. A. Marlett. 1981. The viscosity interaction of barley beta-glucan with Trichoderma viride cellulase in the chick intestine. Poult. Sci. 60:1043–1048.

Table 4.1. Ingredient composition of the corn-soybean meal diet fed from 1 to 6 d of age

Ingredient	%
Corn	54.87
Soybean Meal, 48% CP	34.09
Soybean oil	2.80
Defluorinated phosphate	1.24
Biofos	0.40
Limestone	0.80
L-Thr-98.5%	0.13
DL-Met	0.35
Lysine HCl	0.23
UGA vitamins[1]	0.25
Salt	0.20
UGA trace minerals[2]	0.15
Filler	4.48
Calculated nutrient composition[3]	
ME energy (Kcal/kg)	2,975
Protein (%)	21.0
Total Crude Fiber (%)	2.78
Calcium (%)	0.90
Dig. Phosphorus (%)	0.45
Dig. Met (%)	0.65
Dig. TSAA (%)	0.91
Dig. Lys (%)	1.22
Dig. Thr (%)	0.83

[1]Vitamin premix provided the following per kilogram of DSM premix: Vit. A, 2,204,586 IU; Vit. D_3, 200,000 ICU; Vit. E, 2,000 IU; Vit. B12, 2 mg; Biotin, 20 mg; Menadione, 200 mg; Thiamine, 400 mg; Riboflavin, 800 mg; d-Pantothenic Acid, 2,000 mg; Vit. B6, 400 mg; Niacin, 8,000 mg; Folic Acid, 100 mg; Choline, 34,720 mg.

[2]Mineral premix includes per kg of premix: Ca, 0.72 g; Mn, 3.04 g; Zn, 2.43 g; Mg, 0.61 g; Fe, 0.59 g; Cu, 22.68 g; I, 22.68 g; Se, 9.07 g.

[3]Values reported as percentages unless noted otherwise.

Table 4.2. Ingredient composition of the semi-purified basal diet

	%
Dextrose	50.00
Corn starch	11.64
Casein, dehydrated	15.00
Pork meat and bone meal	7.35
Corn gluten meal	5.04
Fish meal	0.14
Salt	0.40
DL-Met	0.26
Vitamins	0.25
Minerals	0.15
Limestone	0.27
Sand[3]	9.50
Calculated nutrient composition[4]	
ME energy (Kcal/kg)	3,170
Protein (%)	21.0
Total Crude Fiber (%)	0.0
Calcium (%)	0.90
Dig. Phosphorus (%)	0.50
Dig. Met (%)	0.80
Dig. TSAA (%)	0.91
Dig. Lys (%)	1.50
Dig. Thr (%)	0.88

[1]Vitamin premix provided the following per kilogram of DSM premix: Vit. A, 2,204,586 IU; Vit. D_3, 200,000 ICU; Vit. E, 2,000 IU; Vit. B12, 2 mg; Biotin, 20 mg; Menadione, 200 mg; Thiamine, 400 mg; Riboflavin, 800 mg; d-Pantothenic Acid, 2,000 mg; Vit. B6, 400 mg; Niacin, 8,000 mg; Folic Acid, 100 mg; Choline, 34,720 mg.

[2]Mineral premix includes per kg of premix: Ca, 0.72 g; Mn, 3.04 g; Zn, 2.43 g; Mg, 0.61 g; Fe, 0.59 g; Cu, 22.68 g; I, 22.68 g; Se, 9.07 g.

[3]Sand was replaced with 9% of the adequate soluble to insoluble fiber ratio as shown in table 4.3.

[4]Values reported as percentages unless noted otherwise.

Table 4.3. Fiber ratios in experimental treatments[1]

TRT	Ratio (soluble: insoluble)	Soluble[2], %	Insoluble[2], %	Total added fiber[3]
1	--	--	2	2
2	4:1	7.2	1.8	9
3	2:1	6	3	9
4	1:1	4.5	4.5	9
5	1:2	3	6	9
6	1:4	1.8	7.2	9

[1]Each fiber ratio was added to the semi-purified basal diet separately and fed from d7 to 21 of age
[2]Percentage of the diet that replaced sand
[3]Total fiber added is the sum of soluble + insoluble fiber

Table 4.4. Effects of soluble to insoluble fiber ratios on growth performance of male broilers reared to 21 d of age[1]

	Soluble to insoluble fiber ratio[2]							
Item[3]	CTL T1	4:1 T2	2:1 T3	1:1 T4	1:2 T5	1:4 T6	SEM[4]	*P*-value
BWG (g), d14	76	65	67	66	66	70	4	0.307
FI (g), d14	195	190	181	190	193	192	7	0.436
FCR, d14	2.58	3.09	2.74	2.9	2.94	2.81	0.2	0.217
BWG (g), d21	102.5^{ab}	81^{c}	96^{abc}	85^{bc}	89^{abc}	103.3^{a}	5	<0.001
FI (g), d21	371	355	356	349	370	365	14	0.399
FCR, d21	3.62^{ab}	4.37^{b}	3.76^{ab}	4.11^{ab}	4.18^{ab}	3.55^{a}	0.2	<0.01

[1]Values are the least-square means of 6 replicate pens per treatment with 12 birds per cage. Where applicable, means were separated using Tukey's Honestly Significant Difference Test
[2]Diets were formulated using different amounts of solka floc® (SF) or pectin to achieve the different ratios. All dietary treatments (except the control) were added 9% dietary fiber.
[3]Mortality-corrected body weight gain (BWG), feed intake (FI), and feed conversion ratio (FCR) per bird.
[4]SEM= largest pooled standard error of the pairwise mean comparison
[a-b]Means within a row not sharing a common superscript differ significantly ($P < 0.05$)

Table 4.5. Effects of soluble to insoluble fiber ratios on digestive organ growth of male broilers reared to 21 d of age[1]

		Soluble to insoluble fiber ratio[2]							
		CTL	4:1	2:1	1:1	1:2	1:4		
	Item, %	T1	T2	T3	T4	T5	T6	SEM[3]	*P*-value
Day 14	Gizzard	3.75	3.67	3.54	3.44	3.61	3.68	0.2	0.900
	Liver	4.04	3.89	3.99	4.14	4.23	4	0.29	0.972
	Ceca	0.77	0.94	0.71	0.84	0.91	0.71	0.11	0.578
	SI[4]	7.62	7.94	7.57	7.79	7.74	7.2	0.30	0.605
Day 21	Gizzard	3.3	3.46	2.81	3.07	3.02	3.86	0.31	0.210
	Liver	3.9	4.63	3.44	4	4.08	3.85	0.39	0.425
	Ceca	1.04	1.15	0.86	1.3	1	1.28	0.18	0.391
	SI[4]	6.38	7.81	7.71	6.72	6.42	6.57	0.53	0.139

[1]Values are the least-square means of 6 replicate pens per treatment with 12 birds per cage. Where applicable, means were separated using Tukey's Honestly Significant Difference Test

[2]Diets were formulated using different amounts of solka floc® (SF) or pectin to achieve the different ratios. All dietary treatments (except the control) were added 9% dietary fiber.

[3]SEM= largest pooled standard error of the pairwise mean comparison

[4]SI= small intestine

[a-b]Means within a row not sharing a common superscript differ significantly ($P < 0.05$)

Table 4.6. Villi height, crypt depth, and villi:crypt ratio from the duodenum, jejunum, and ileum of male broilers reared to 21 d of age[1]

		Soluble to insoluble fiber ratio[2]							
		CTL	4:1	2:1	1:1	1:2	1:4		
	Item	T1	T2	T3	T4	T5	T6	SEM[3]	*P*-value
Duodenum	Villi, µm	1710^{ab}	1743^{a}	1711^{ab}	1745^{a}	1533^{b}	1743^{a}	56	0.006
	Crypt, µm	171^{a}	165^{a}	174^{a}	161^{ab}	145^{b}	170^{a}	9	0.023
	Ratio, µm	10.43	11.00	10.45	11.21	11.1	10.43	0.56	0.686
Jejunum	Villi, µm	927^{bc}	1244^{a}	1113^{ab}	1235^{a}	1269^{a}	888^{c}	59	<0.001
	Crypt, µm	164	146	154	149	154	153	11	0.843
	Ratio, µm	6.4^{bc}	8.94^{a}	7.51^{abc}	8.79^{ab}	8.83^{a}	6.28^{c}	0.67	<0.001
Ileum	Villi, µm	793^{a}	607^{b}	730^{a}	782^{a}	767^{a}	826^{a}	0.31	<0.001
	Crypt, µm	138^{b}	140^{b}	154^{ab}	175^{a}	153^{ab}	129^{b}	8	<0.001
	Ratio, µm	5.98^{ab}	4.57^{b}	5.0^{b}	4.53^{b}	5.45^{ab}	6.59^{a}	0.38	<0.001

[1]Values are the least-square means of 6 replicate pens per treatment with 12 birds per cage. Where applicable, means were separated using Tukey's Honestly Significant Difference Test
[2]Diets were formulated using different amounts of solka floc® (SF) or pectin to achieve the different ratios. All dietary treatments (except the control) were added 9% dietary fiber.
[3]SEM= largest pooled standard error of the pairwise mean comparison
[a-b]Means within a row not sharing a common superscript differ significantly ($P < 0.05$)

Table 4.7. Effects of soluble to insoluble fiber ratios on nutrient digestibility and intestinal viscosity on 21 d of age[1]

	Soluble to insoluble fiber ratio[2]							
Item	CTL T1	4:1 T2	2:1 T3	1:1 T4	1:2 T5	1:4 T6	SEM[3]	*P*-value
DM, %	81.97[b]	81.71[b]	82.79[ab]	85.77[a]	82.30[ab]	85.77[a]	0.84	0.012
AME, kcal/kg	3051[b]	3091[b]	3124[b]	3257[a]	3095[b]	3275[a]	25	<0.01
CP, %	80.69[a]	66.57[c]	71.85[bc]	78.05[ab]	74.45[ab]	80.72[a]	0.90	0.005
Viscosity d14, mPas	6.15	13	10.21	7.03	8.75	8.37	2.70	0.441
Viscosity d21, mPas	3.78[c]	11.6[a]	9.19[ab]	7.90[ab]	5.86[bc]	4.6[bc]	0.63	0.009

[1]Values are the least-square means of 6 replicate pens per treatment with 12 birds per cage. Where applicable, means were separated using Tukey's Honestly Significant Difference Test
[2]Diets were formulated using different amounts of solka floc® (SF) or pectin to achieve the different ratios. All dietary treatments (except the control) were added 9% dietary fiber.
[3]SEM= largest pooled standard error of the pairwise mean comparison
[a-b]Means within a row not sharing a common superscript differ significantly ($P < 0.05$)

CHAPTER 5

EFFECTS OF FIBER TYPE, PARTICLE SIZE, AND INCLUSION LEVEL ON THE GROWTH PERFORMANCE, DIGESTIVE ORGAN GROWTH, INTESTINAL VISCOSITY, INTESTINAL MORPHOLOGY, AND GENE EXPRESSION OF BROILERS[1]

[1]Tejeda, O. J. and W. K. Kim. Submitted to Poultry Science.

Abstract

The aim of this study was to evaluate the effect fiber type, particle size, and inclusion level on the performance parameters, intestinal development and gene expression in broiler chickens. A total of 648 one-day old Cobb® male broilers were randomly assigned to a control diet and 8 other dietary treatments divided in 2 fiber types (cellulose vs soyhulls), 2 particle sizes (100 and 600 µm), and 2 inclusion levels (4% and 8% crude fiber). Birds were reared to 21 days of age in battery cages (n=6 replicates). Growth performance parameters and intestinal viscosity were measured on days 7, 14, and 21. On day 14 and 21, digestive organ weights were recorded for analyses of organ growth. On day 21, intestinal samples were taken for analyses of histology, and jejunal mucosas were collected for analyses of nutrient transporters. Data were analyzed as a 2 × 2 × 2 factorial design using JMP® 2021. Treatments where compared against the control group using one-way analyses of variance, whereas the main effect interactions were evaluated as a factorial excluding the control group to be able to assess the effect of the independent variables without the variability introduced by the control group. Groups fed 8% crude fiber from cellulose (8% CL) had the lowest weight gain regardless of the particle size ($P < 0.01$). The control group had the highest feed intake among treatments ($P < 0.01$). Groups fed 8% crude fiber from soyhulls (8% SH) with a coarse particle size had the heaviest relative gizzard weight among treatments ($P = 0.045$). Groups fed 8% SH had the heaviest small intestine weights regardless of the particle size ($P = 0.009$). No differences were observed in the relative weights of the ceca. The highest viscosity was observed in the group fed 8% SH with a fine particle size ($P < 0.001$). The group fed 4% SH with a coarse particle size had the longest duodenal villus ($P < 0.001$). The shortest jejunal villus height was observed in the group fed 8% CL with a fine particle size ($P < 0.001$). Ileal villus was highest in groups fed high cellulose levels regardless of the particle size ($P < 0.001$). The highest digestibility

of dry matter was observed in the group 4% SH with fine particle (P = 0.017). The group 4% CL with fine particle had the highest digestibility of crude protein (P = 0.033). The highest expression of peptide transporter 1 was observed in the group fed 8% CL with a coarse particle size (P = 0.008). In conclusion, fiber type, particle size, and inclusion levels are important factors in the regulation of intestinal morphology, viscosity, nutrient transporters, and growth performance.

Key words: fiber, particle size, inclusion level, intestinal morphology, broiler

Introduction

Current tendencies to incorporate cheaper feed ingredients in the formulation of poultry diets have led to the adoption of fibrous feed ingredients. Different feedstuffs vary in the type, amount, and proportions of dietary fiber (DF) that they contain (Jaworski et al., 2015; Knudsen, 2014; Nguyen et al., 2019) which provides a wide array of potential physiological and nutritional implications when used in broiler diets (Hetland et al., 2003; Jiménez-Moreno et al., 2016; Owusu-Asiedu et al., 2006). Dietary fiber has been associated with changes in growth performance (Hetland and Svihus, 2001; Jiménez-Moreno et al., 2016), intestinal morphology (Sittiya et al., 2020; Sklan et al., 2003), and nutrient digestibility (Cao et al., 2003; Tejeda and Kim, 2020) that are generally ignored when using fibrous by-products as feed ingredients.

Fiber type, amount used, and particle size are the most crucial factors to bear in mind when using dietary fiber as a functional nutrient in the nutrient matrix (Hetland et al., 2004; O'Dell et al., 1959; Tejeda and Kim, 2021). Fiber type can be explained in terms of the ability of the fibrous components to form interactions with water molecules (i.e. soluble or insoluble) (Chaplin, 2003). Viscous soluble fibers have been associated with impairment in growth performance due to disruption of normal enzymatic activity and nutrient digestibility (Hetland et

al., 2004; Saki et al., 2011). Insoluble fibers used in low amounts (i.e. 3-5%) have been shown to modulate intestinal morphology and nutrient utilization (Chiou et al., 1996; Tejeda and Kim, 2020). Particle size seems to have a paramount role in modulation of intestinal motility and subsequently nutrient utilization (Kheravii et al., 2018). This ability has been reported to be important along the different portions of the gastrointestinal tract for both, big and small particle sizes (Amerah et al., 2007).

It has been clearly demonstrated the that dietary fiber modulates intestinal development (Sadeghi et al., 2015; Sklan et al., 2003) and general nutrient metabolism (Georgieva et al., 2014; Hetland et al., 2004; Kheravii et al., 2018) depending on the type and amount incorporated in the diet. However, little is known about the role that particle size plays when using different fiber types an inclusion level. Therefore, the objective of this study was to evaluate the effects of two sources of fiber (cellulose and soybean hulls), two inclusion levels (4% and 8% crude fiber), and two particles sizes (100µm and 600µm) on the growth performance, digestive organ growth, intestinal viscosity, intestinal morphology, nutrient digestibility and gene expression of broilers.

Materials and methods

General Procedures

The experiment was approved by the Institutional Animal Care and Use Committee (IACUC) of the University of Georgia (Athens, Georgia, United States). A total of 648, one-day-old male Cobb500 broiler chicks were allocated in a completely randomized factorial designed with nine dietary treatments and 6 replicates of 12 birds each. There were three main factors namely, fiber type (cellulose and soyhulls), inclusion levels (4 and 8% crude fiber) and particle size (100 and 600µm). The chicks were allocated in 54 cages equipped with one drinker and one feeder, providing *ad-libitum* access to water and mash feed from 1 to 21 days of age.

Temperature and lighting program followed the recommendation of Cobb Broiler Management Guide (Cobb-vantress, 2018).

Dietary Treatments

All diets were corn and soybean meal-based formulated to meet the nutrient requirements specified by Cobb500 performance and nutritional guide (Cobb-Vantress, 2018). The control diet was formulated to contain 2% crude fiber (CF). The control diet was used as a basal diet to which purified cellulose (**CL**: 99% cellulose, Solka floc®, Skidmore, Schollcraft, MI) was added as a source of CF by replacing an inert filler (sand) to achieve 4 and 8% CF (4% CL and 8% CL) in the diets. Solka floc® 100-fcc and solka floc® 900-fcc with an average particle size of 100 and 600 µm, respectively, were added separately to their adequate dietary treatment as source of purified cellulose. The rest of experimental diets were added increasing amounts of soyhulls (**SH**) to achieve 4 and 8% CF (4% SH and 8% SH). Particle sizes averaging 100 and 600 µm of soyhulls were obtained using a machine mill with different screen sizes (Fitzpatrick model M comminuting machine mill, the W. J. Fitzpatrick company, Chicago, Illinois, USA). Proximate analyses of soyhulls was conducted to measure the gross nutrient content (**Table 5.1**). For amino acids and apparent metabolizable energy corrected for nitrogen (AME_n), the nutrient matrix composition used for soyhulls was obtained using cecectomized roosters at the poultry research center at the University of Georgia (**Table 5.2**). Diets were provided as mash during the entire rearing period (0-21 d). All diets were isonitrogenous and isocaloric and are shown in Table 5.3. For ileal nutrient digestibility determination, chromic oxide (Cr_2O_3, Sigma Aldrich, St. Louis, MO) was added at 0.3% as an indigestible marker to all diets.

Growth Performance and Organ Weights

The birds and feed were weighed weekly per cage to determine mortality-corrected body weight gain (BWG), mortality-corrected feed intake (FI), and mortality-corrected feed conversion ratio (FCR) and results are presented per week. Mortality was recorded twice daily. For organ growth analyses, empty gizzard, small intestine and ceca were obtained from one average bird per cage (n = 6 per treatment) and weighed to determine the relative organ weight on d 14 and 21.

Intestinal Morphology

On d 21, samples from the mid-duodenum, jejunum and ileum (~ 2cm long) were collected from one average bird per replicate cage (n = 6 per treatment). Intestinal contents were flushed with phosphate-buffered saline (PBS) and intestinal sections were stored in 10% neutral-buffered formalin and left in solution for a minimum period of 48 hours for tissue fixation. During slide preparation, increasing amounts of ethanol were used to dehydrate the tissues, then diaphanized in dimethylbenzene, and fixed in paraffin. Finally, tissue sections with a thickness of 4-µm were set on slides and were stained using Hematoxylin and Eosin (H&E) procedures. Pictures were taken using a light microscope (10x eyepiece and 1.6x magnification; Leica DC500 camera, Leica Mycrosystems Inc., Buffalo Groove, IL). Measurements for villi height and crypt depth were taken using ImageJ software (Image Processing and Analysis in JAVA – ImageJ 1.52r, National Instituted of Health).

Intestinal Viscosity

On 7, 14 and 21 days of age, one bird per cage was randomly selected and euthanized, and intestinal digesta was collected from the Meckel's diverticulum to the ileocolonic junction.

Fresh digesta were centrifuged at 12,600 × g for 5 minutes and the supernatants were collected for viscosity measurements using a cone and plate Brookfield DV-II + Programmable viscometer at 10 rpm using a CPE-40 spindle (Brookfield engineering laboratories, Inc, Middleboro, MA, USA). A water bath control connected to the cone was used to keep the temperature of the samples at 40 ºC.

Nutrient Digestibility

On d 21, six birds per replicate cage were euthanized, and ileal digesta were collected from two-thirds of the distal ileum (from Meckel's diverticulum to about 1 inch anterior to ileocecal junction). The digesta samples were dried for analyses of dry matter, crude protein and energy. The chromium oxide concentration was measured according to Dansky and Hill (1952), and gross energy was evaluated using a bomb calorimeter (IKA Calorimeter C1, IKA Works Inc., Wilmington, NC) at the University of Georgia. The crude protein (N × 6.25) was analyzed at the University of Georgia using a LECO nitrogen analyzer (LECO, St. Joseph, MI, USA). The apparent ileal digestibility (AID) of dry matter, crude protein, and apparent metabolizable energy (AME) were calculated using the following equation:

$$AID, \% = 100\left[1 - \left(\frac{Cr_{feed}}{Cr_{dig}}\right) \times \left(\frac{Nutrient_{dig}}{Nutrient_{feed}}\right)\right]$$

where Cr_{feed} and Cr_{dig} are the chromium dioxide in feed and ileal digesta, respectively; and $nutrient_{dig}$ and $nutrient_{feed}$ are the nutrient in ileal digesta and feed, respectively.

Quantitative Reverse-Transcriptase Polymerase Chain Reaction (qRT-PCR)

On d 21, samples from the jejunal mucosas were collected from one randomly selected bird per cage, snap-frozen in liquid nitrogen, and stored at -80°C previous to analysis. Jejunal mucosa samples were used to analyze the expression of Na+-dependent glucose transporter 1 (SGLT-1), and peptide transporter 1 (Pept-1) genes, using quantitative reverse-transcriptase

polymerase chain reaction (**qRT-PCR**). Total RNA was extracted from the samples previously stored at -80°C using QIAzol® Lysis Reagent (Qiagen, Germatown, MD) according to the manufacturer's instruction. After extraction, RNA quantity and purity were determined using Nanodrop 1000 spectrophotometer (Thermo Fisher Scientific, Pittsburgh, PA). The cDNA was synthesized from total RNA and subsequently diluted to 10 ng/µl for qRT-PCR analysis. Glyceraldehyde 3-phosphate dehydrogenase (GAPDH) was used as the housekeeping gene. The forward and reverse primers for the genes are shown in Table 5.4. The qRT-PCR was performed on an Applied Biosystems StepOnePlus™ (Thermo Fisher Scientific, Waltham, MA) with iTaq™ Universal SYBR Green Supermix (BioRad, Hercules, CA) using the following conditions: 95°C for 15 s, 58°C for 20 s, and 72°C for 15 s during 40 cycles for GAPDH; 95°C for 15 s, 60°C for 20 s, and 72°C for 15 s during 40 cycles for Pept-1; and 95°C for 15 s, 58°C for 20 s, and 72°C for 15 s during 40 cycles for SGLT-1. Samples were ran in duplicate and relative gene expression data were analyzed using the $2^{-\Delta\Delta Ct}$ method (Livak and Schmittgen, 2001). The mean ΔCt of control group was used to calculate the ΔΔCt value.

Statistical Analyses

Fiber type, particle size and inclusion level were the fixed effects in the model. Pen was used as the experimental unit for growth performance and nutrient digestibility; bird was used as the experimental unit for organ growth, intestinal morphology, intestinal viscosity, and gene expression. Data were analyzed as a completely randomized block design with 8 treatments organized as 2 × 2 × 2 factorial. One-way analyses of variance was used to determine the effect of fiber inclusion compared to the control group, whereas the main effect interactions were evaluated excluding the control group to be able to assess the effect of the independent variables

without the variability introduced by the control group. The main effects model used for statistical analyses is as follows:

$$Y_{ij} = \mu + \alpha_i + \beta_j + \delta_t + \alpha\beta\delta_{ijt} + \varepsilon_{ijt}$$

where Y_{ij} represents the value for each random variable; μ is the overall mean; α_i, β_j, δ_t, and $\alpha\beta\delta_{ijt}$ are the fiber type, inclusion level, particle size, and their interactions, respectively such that $\Sigma\alpha_i=0$; and the random errors ε_{ijt} are identically and independently normally distributed with a mean 0 and a variance σ. All statistical procedures were performed using JMP® Pro (SAS Institute, 2021). In case of significant differences, means were separated using the Tukey's test HSD option. For all hypothesis tests, statistical significance was considered at $P < 0.05$.

Results

Growth performance

The results for growth performance are presented per week in Table 5.5. The upper portion of the table includes the control group, and the second portion of the table includes the main effects and their interactions only, without the control group. On day 21, the control group had the heaviest weigh gain during the rearing period but did not differ from the treatments containing 4% CF regardless of the fiber source ($P > 0.05$). However, the treatments fed 8% CL had the lowest weight gain at the end of the experiment ($P < 0.001$). The control group had the highest feed intake during the entire rearing period ($P < 0.05$). The groups fed 4% CF with a fine particle size had lower FCR on 7 days of age ($P = 0.015$). However, such differences disappeared in the rest of the experiment ($P > 0.05$). The results from the main effects show that fiber type did not affect significantly any of the growth performance parameters ($P > 0.05$). The statistics for the main effects indicate that fine particle size (100 µm) increased the weight gain on days 7 and 14 and improved the FCR on day 7 compared to the coarse particle size ($P =$

0.021); however, such differences disappeared at the end of the experiment ($P > 0.05$). Fiber level affected all the growth performance parameters where the highest fiber level (8% CF) resulted in lower weigh gain, lower feed intake, and poorer FCR compare to the groups fed 4% CF ($P < 0.05$). The interaction, fiber type × particle size, was significant on day 7 for weight gain, where coarse SH improved weight gain compared to the fine soyhulls, and coarse CL decreased weight gain compared to fine CL ($P = 0.0021$). Particle size × level interaction on day 7 for feed intake shows that fine particles at low levels had higher feed intake compared to coarse particles at high levels on day 14 ($P = 0.023$). Three-way interaction among main effects on day 14 and 21 for feed intake shows that fine soyhulls at the low level had the heaviest weight gain, whereas the lowest weight gain was for the group fed coarse and high levels of CL ($P = 0.032$). Three-way interactions also indicate that the group fed coarse and high levels of CL had the highest feed intake among dietary treatments ($P = 0.044$). However, no differences were observed in the FCR ($P > 0.05$). At the end of the experiment, the mortality was higher for groups fed soyhulls, specially treatments given the highest amounts of soyhulls ($P < 0.05$).

Intestinal Histomorphology

Results for intestinal morphology are shown in Table 5.6. Duodenal villus was highest for the treatment fed 4% SH with a coarse particle size, whereas the shortest duodenal villus was observed in the group fed 8% CL with a fine particle size ($P < 0.001$). The control group had the deepest duodenal crypt among the treatments ($P = 0.034$). The smallest duodenal villus to crypt ratio was observed in the group fed 8% CL with a fine particle ($P < 0.01$). The control group and the group fed 8% SH with a fine particle had the highest and shortest jejunal villus, respectively ($P < 0.01$). No differences were observed in the jejunal crypts among the dietary treatments ($P = 0.2015$). The group fed 8% SH with a fine particle size had the smallest jejunal villus to crypt

ratio among dietary treatments ($P < 0.01$). Ileal villus was highest in the control group compared to the rest of the dietary treatments ($P < 0.001$). Ileal crypt depth was highest for the groups fed coarse SH regardless of the inclusion level ($P < 0.001$). Ileal villus to crypt ratio was highest for 8% CL with coarse particle and lowest for all treatments containing SH regardless of the inclusion level or particle size. The results for the mean effects show that SH-fed treatments had highest crypt depth and shorter ileal villus and ileal villus to crypt ratio compared to groups fed CL ($P < 0.05$). Coarse particle size increased duodenal and jejunal villus height and crypt depth, ($P < 0.01$). High fiber level decreased duodenal villus height, crypt depth, and their ratio, and jejunal villus height and crypt depth; however, it increased jejunal villus to crypt ratio and ileal villus height and ileal villus to crypt ratio ($P < 0.05$). The interaction, type × particle size, was significant for duodenal villus height where the groups fed SH with fine particle had higher duodenal villus compared to the groups fed CL with fine particle ($P = 0.0301$). The interaction, type × inclusion level, shows that 4% SH-fed groups had higher duodenal villus height compared to those fed CL-4%, and shorter jejunal villus and villus to crypt ratio ($P < 0.05$). Three-way interaction, type × particle size × inclusion level, shows that coarse SH at a low level had the highest jejunal crypt depth, and the 8% SH with coarse particle had the highest villus to crypt ratio ($P < 0.05$).

Digestive Organ Growth

The results for organ growth are shown in Table 5.7. The groups fed the diets containing SH had the heaviest gizzard on day 14 but only the group fed SH at 8% CF with coarse particle maintained such increased weight until day 21 ($P < 0.05$). The groups containing 4 and 8% CF as SH in both particle sizes had the heaviest small intestines weight compared to the rest of the treatments on day 21 ($P = 0.009$). No statistical differences were observed in the ceca weights

relative to body weight among the dietary treatments ($P > 0.05$). The results of the main effects show that SH-fed groups had a heavier gizzard on day 14 compared to the CL-fed groups ($P = 0.006$). Fiber type did not significantly affect small intestine or ceca relative weights ($P > 0.05$). Fiber with coarse particle (i.e. 600 µm) increased the relative weights of the gizzard on day 21 ($P = 0.026$) compared to fine particle (i.e. 100 µm). The groups fed 8% CF had heavier gizzard and intestines on day 21 ($P < 0.05$) compared to those fed 4% CF regardless of fiber type. The interaction, fiber type × particle size, was significant; fine CL decreased the weights of the gizzard on day 14 ($P = 0.002$). However, this effect disappeared on day 21. Fiber type × level interaction indicates that the group fed the high level of SH increased the weight of small intestines, whereas the low level of SH had the lowest intestine weight ($P = 0.047$). No other significant interactions were observed among the main effects ($P > 0.05$).

Intestinal Viscosity

Results for intestinal viscosity are shown in Table 5.8. On day 7, the group fed 8% CF as SH with a coarse particle size had the highest intestinal viscosity, and the lowest was for the group fed 8% CF with coarse CL ($P = 0.045$). On day 14, the groups containing 8% CF as SH had the highest intestinal viscosity regardless of the particle size ($P < 0.001$). However, on day 21, the group having 8% CF as SH with a fine particle size had the highest intestinal viscosity ($P < 0.001$). Results from the main effects show that viscosity was higher in the groups fed SH compared to those fed CL on days 7, 14, and 21 ($P < 0.05$). The main effects show that particle size was not statistically significant in affecting intestinal viscosity ($P > 0.05$). Higher fiber inclusion increased the intestinal viscosity on days 14 and 21 ($P < 0.05$). The interaction. fiber type × level, was significant where the 8% SH-fed group had the highest intestinal viscosity on

days 7, 14, and 21 ($P < 0.01$). No other significant interactions were observed among the main effects ($P > 0.05$).

Nutrient Digestibility

The results for nutrient digestibility are shown in Table 5.9. In the present experiment, all diets were formulated to be isonitrogenous and isocaloric. Statistical differences were observed in the digestibility of dry matter where the group fed 4% SH with fine particle had the highest DM digestibility, whereas the treatment fed 8% SH with coarse particle had the lowest DM digestibility ($P = 0.0169$). The digestibility of crude protein was improved for the group fed 4% CL with a fine particle and was worst for the groups fed 8% SH ($P = 0.0326$). No statistical differences were observed in the digestibility of energy ($P > 0.05$). The results from the main effects indicate that particle size is an important factor in the modulation of nutrient digestibility where the smaller particle (100μm) increased nutrient digestibility compared to the larger one (600μm) ($P < 0.05$). Inclusion level significantly affected the digestibility of DM where higher inclusions reduced such parameter ($P = 0.007$).

Gene Expression

The results for gene expression of nutrient transporters are shown in Table 5.10. No significant differences were observed in the expression of SGLT-1. However, the expression of Pept-1 was higher for the group fed 8% CL with coarse particle compared to the control group ($P = 0.008$). The results from the main effects show that none of the individual main effects have a significant impact in the expression of nutrient transporters ($P > 0.05$). However, the interaction, fiber type × particle size, shows that coarse particle of CL increased the expression of Pept-1 compared to coarse particle of SH ($P = 0.0154$).

Discussion

Growth Performance

To evaluate the impact of fiber type, inclusion level, and particle size, all diets were formulated to be isonitrogenous and isocaloric. However, despite the fact that diets with similar nutrient content were used, there were differences in growth performance among the dietary treatments. Treatments having low fiber inclusion (4% CF) did not differ from the control group. This is in accordance with other researchers that have reported that small inclusions of dietary fiber do not affect negatively the growth performance of broilers (Amerah et al., 2009; Sacranie et al., 2012). However, the treatments fed 8% CF using CL with regardless of particle size had the worst weight gain among dietary treatments whereas those fed 8% CF as SH with a course particle size did not differ from those fed 4% CF. This can be attributed to the differences in fiber types between CL and SH. Cellulose (CL) contains a tertiary structure linked together by an extensive number of hydrogen bonds, providing stability, low aqueous solubility and mostly resistance to acid hydrolysis (Festucci-Buselli et al., 2007) which might encapsulate nutrients in the upper digestive tract and reduce the break down at the level of the gizzard leading to interference in the breakdown of other nutrients. The control group had the highest feed intake compared to the other fiber-containing treatments during the entire rearing period in the current study. Other studies also reported that broilers given choice feeding between control and diet containing rice hulls had a lower feed intake compared to the control group (González-Alvarado et al., 2008; Sadeghi et al., 2015). However, other researchers have pointed out the ability of broilers to increase feed intake as a means to compensate for the nutrient dilution when using dietary fiber (Amerah et al., 2009; Sacranie et al., 2012). This is associated to the differences in nutrient content in experimental diets (no isocaloric diets). In our experiment, all diets were

isocaloric and isonitrogenous; thus, increases in feed intake should not be expected. The reduction in feed intake of the treatments containing 8% cellulose in either particle size and 8% SH with a particle size of 100 µm was not expected and can be attributed to the impact of dietary fiber on intestinal motility and passage rate. In contrast to these results, Amerah et al. (2009) reported an increased in feed intake when the control diet was diluted with cellulose in the ratio 6:10. In our experiment, however, filler (sand) was replaced with the adequate level of cellulose to maintain the same nutrient content. Differences in FCR were observed just in the first week of the rearing period where the groups fed 4% CF with a particle size of 100 µm had the lowest FCR regardless of the fiber source. The opposite was true for the groups fed 8% CF which had the poorest FCR. However, such differences were not seen in the rest of the experiment. Birds fed cellulose (CL) at 8% had the lowest weight gain irrespective of the particle size. In the case of SH, the group fed 8% CF as SH with a coarse particle did not differ from the groups fed 4% CF. The results from the main effects excluding the control group show that fine particle size of fiber improved weigh gain on days 7 and 14 and FCR on day 7. However, such differences disappeared at the end of the experiment. Similar to these results, Donadelli et al. (2019) found that fine particles reduced the FCR when using different fiber types. Other researchers have suggested that insoluble fiber with coarse particle, in some cases, can help in the improvement of growth performance by modulating intestinal functionality (Choct, 2015). In our experiment, it is important to mention that for CL the groups fed the same amounts of fiber (i.e. 4% or 8%) had similar weight gain in both particle sizes. However, for SH it was observed better results when SH is provided in a coarse particle size compared to the fine particle size. These differences might be associated to the fiber matrix found of soyhulls which is composed of pectins, cellulose, and hemicellulose (Stein and Parsons, 2008) which interact differently in the

gastrointestinal tract compared to purified cellulose. Groups fed 8% CF had lower weight gain, feed intake and higher (poorer) FCR compared to those fed 4% SH. In fact, the mortality for groups fed 8% soyhulls was higher. The presence of soluble fiber in soyhulls are the main reason behind increases in mortality, just when given in high amounts as observed in this experiment. Other results have previously been reported that high levels of dietary fiber reduce growth performance parameters in diets with the same nutrient level (Sklan et al., 2003) as well as diets where fiber has been replaced without nutrient adjustment (Hetland and Svihus, 2001), which indicates the ability of high fiber levels to encapsulate the nutrients making them unavailable for absorption (Hetland et al., 2004).

The interaction, type × particle size, was significant on day 7, where coarse SH improved weight gain compared to fine soyhulls. Furthermore, coarse CL decreased weight gain compared to fine CL. These results point out the importance of particle size based on the type of fiber used in the diets. Particle size × level interaction on day 7 for feed intake shows that fine particles at low levels had higher feed intake compared to coarse particles at high levels in the present study. Different researchers have indicated the potential of coarse fibers to modulate digesta passage rate and nutrient digestion by increasing the retention time in the upper digestive tract (i.e. gizzard) (Gonzalez-Alvarado et al., 2007; Hetland et al., 2004). This might explain the higher intake for diets containing fine particle size which fail in stimulating retention of feed components at the level of the gizzard. In the present study, three-way interaction among the main effects on day 14 and 21 for feed intake shows that fine SH at the lower level had the heaviest weight gain, whereas the lowest weight gain was for the group fed coarse and high levels of CL. In chicks, it has been clearly demonstrated that nutrient digestibility increases from 58% to up to 90% when coarse particles are ground to finer particles (Mitchell et al., 1972). It is

important to highlight that this is true for nutrient-containing feedstuffs. In the present experiment, SH and CL were used as sources of fiber. SH is a substantial source of fiber (75% NDF) but also contains 16% crude protein and 658 kcal/kg ME (Table 5.1); on the other hand, CL is a purified source of cellulose (99% cellulose) which explains the reason behind the differences in performance when using fine particle size of soyhulls at low levels (4% CF). Three-way interactions also show that the group fed coarse and high levels of cellulose had the highest feed intake among the dietary treatments despite the fact that all diets were isocaloric and isonitrogenous; however, this was not true for the group fed SH, indicating that high levels of pure insoluble fibers can modulate feed intake in broilers as reported for other researchers (Donadelli et al., 2019; Hetland et al., 2003).

Digestive Organ Growth and Digesta Viscosity

All groups containing CL had a similar relative weight of the gizzard irrespective of the inclusion level or particle size. This has been seen by other researchers when using small amounts (< 5%) of cellulose (Cao et al., 2003). However, in the present study, when comparing between fiber sources, the group fed 8% CF as SH with a coarse particle size had the heaviest gizzard relative weight, whereas the group fed fine SH at 4% CL had the lowest one. These results are in accordance with other study reporting that chickens fed fine particles developed smaller gizzard compared to those fed coarser particles (O'Dell et al., 1959). This is because the gizzard functions as a sieve that retains and grinds coarse particles until they have achieved a determined size before moving to the small intestine (Hetland et al., 2004). Therefore, the presence of fine particles fails to stimulate the muscles of the gizzard, resulting in poorer gizzard development. It was also observed in the present experiment that the groups containing 8% CF as SH had the heaviest small intestine weight (including duodenum, jejunum, and ileum), irrespective of the particle size,

compared to the rest of the treatments on day 21. This could be attributed to the increase in intestinal viscosity observed in the present experiment for SH-containing diets during the entire rearing period when fed at the level of 8% CF. This is because the carbohydrate portion of SH is made up of 30% pectins (Stein and Parsons, 2008) which increases intestinal viscosity, reducing the passage rate of the digesta, and subsequently provoking the growth of the small intestine as a means to offset the changes in volume caused by the accumulation of feed in such organ (Owusu-Asiedu et al., 2006).

In the present study, the interactions among the main effects were observed; the increase of fine soyhulls in the diet caused significantly higher digesta viscosity compared to coarse SH, and fine and coarse CL. This is because the interaction of fiber with water is determined not only by fiber type (i.e. soluble or insoluble) but for its physical properties as well, where smaller particle size can increase water absorption due to higher surface area available to interact with water molecules (Strange and Onwulata, 2002). Interestingly, no significant differences were observed in the relative weights of the ceca. Similar to these results, Gonzalez-Alvarado et al. (2007) did not observed significant differences in the ceca weights of broilers fed 3% soyhulls compared to the control group; however, birds fed insoluble fiber (oat hulls) did have a lower ceca weight compared to the control group. This contrast could be attributed to the fact that these authors used oat hulls as source of insoluble fiber which contains other non-cellulosic components including lignin, protein, and fat (Welch et al., 1983), resulting in different results. From the main effects it is clear that fiber with coarse particle (i.e. 600 µm) increased the relative weights of the gizzard on day 21 compared to fine particle (i.e. 100 µm). This was more pronounced in the diets containing SH as the source of fiber. This can be attributed to the fact that natural fibers have a higher level of polymerization (Hivechi and Bahrami, 2016) resulting

in higher stimulation of the muscles of the gizzard. The fiber level was certainly of influence in the stimulation of the gizzard and also the relative weight of the small intestine. This indicates the need to compensate for the increase of the digesta volume caused by the bulkiness of the fiber particles as observed for other researchers using different fiber types (Hetland et al., 2004; Rezaei et al., 2018; Svihus, 2011).

Intestinal Histomorphology

The treatment 4% SH with coarse particle increased duodenal villus height compared to the group fed 8% CL with fine particle in the current study. This might be associated with the stimulation of the reverse peristalsis provoked by the presence of coarse fiber particles which results in increased villus development (Sacranie et al., 2012). It was also observed that the presence of fine particles in the form of CL reduced the duodenal villus to crypt ratio, indicating a reduction in duodenal functionality. High levels of SH with fine particle size reduced the jejunal villi. However, low levels of SH with fine particle improved jejunal villus to crypt ratio when compared to high levels with fine particle. This inclusion level-dependent differences could be attributed to the excessive abrasive effect of fiber caused when high levels of soyhulls are added to the diet, causing a reduction in villus height as observed by other authors when using other fiber types (Montagne et al., 2003; Sadeghi et al., 2015). This is supported by Tejeda and Kim (2020) who reported that soyhulls fed at 4% crude fiber resulted in improvements in intestinal morphology, but the opposite was true when fed at 6 and 8% CF. Furthermore, the soluble carbohydrates present in SH can also be a reason behind the reduction in jejunal villus height. It has been suggested that soluble carbohydrates can increase the rate of epithelial cell losses, negatively affecting villus growth (Montagne et al., 2003). In the present experiment, it was observed that the highest intestinal viscosity was for groups fed high levels of SH with fine

particle and it can, therefore, be concluded that viscosity played an important role in the atrophy of jejunal villi. The ileal villus was highest for the control group compared to the rest of the treatments. However, the ileal villus to crypt ratio was higher for the 8% CL with coarse particle and smallest for all treatments containing SH. The presence of coarse particles of soluble fiber seems to reduce the development of the ileal villus to crypt ratio. This could be associated to the stimulation of pathogenic bacteria of the undigested carbohydrates at the end of the digestive tract. In accordance with the results in the present experiment, Sadeghi et al. (2015) reported that soluble carbohydrates from sugar beep pulp decreased the ileal villus in broiler compared to the control group. The impairment in the development of ileal villus could, therefore, be associated to the increase in bacterial activity that interferes with the normal intestinal development (Pan and Yu, 2014).

Nutrient Digestibility and Gene Expression

Dietary fibers have been reported to modulate nutrient digestibility in broilers and other poultry species (Amerah et al., 2009; Cao et al., 2003; Hetland et al., 2003; Sacranie et al., 2012). In the present study, the dry matter digestibility was higher for the 4% SH group compared to the 8% SH treatment. From these results, it clear that the digestibility of dry matter is negatively affected by the presence of soluble fibers (i.e. pectins) present in the SH. Similarly, (Silva and Boleli (2013) reported a quadratic decrease in dry matter digestibility with increases of pectin in the diets of broilers. Interestingly, in the present experiment we observed that small amounts of SH increase the dry matter digestibility which could be associated to a slight increase in the retention time that allows for more breakdown of the dry matter. However, the digestibility of crude protein was higher for the 4% CL group with fine particle compared to the 4% SH group with coarse particle. The fact that coarse particles had lower digestibility of crude protein can be

associated to the increase in endogenous amino acid losses caused by higher epithelial cell turnover driven by particle size instead of fiber level (Montagne et al., 2003). In this case, diets with fine CL particles would have lowered endogenous amino acid flow and, therefore, higher protein digestibility. It has been suggested that large particles could slow down the passage rate of digesta at the level of the upper gastrointestinal tract (i.e. gizzard), which would create a prolonged the exposure of nutrients to digestive enzymes, increasing nutrient digestibility (Amerah et al., 2007); however, the effects at the level of the small intestine seem to affect nutrient metabolism differently. Numerical differences in energy digestibility indicate that higher inclusions of fiber reduce energy digestibility despite the fact that diets were formulated to be isocaloric. This might be due to interactions of dietary fiber with other more digestible carbohydrates and/or fat in the diet that renders such nutrients unavailable (Hetland et al., 2004), especially when dietary fiber is given in high amounts. The main effects indicate that coarse particles decreased the digestibility of energy compared to fine particles. Despite the fact that coarse particles can stimulate the upper digestive tract, improving gizzard relative weight, as observed in the present study, the reduction in energy digestibility associated with coarse particles could be attributed to the smaller surface area of coarse particles when compared to smaller particles, which results in a reduction in the accessibility to digestive enzymes (Amerah et al., 2007; Carré et al., 2005).

No significant differences were observed in the expression of jejunal sodium-dependent glucose transporter 1. However, the expression of peptide transporter 1 (Pept-1) was higher for the group fed 8% CL with coarse particle compared to the control group. Interestingly, this group had the poorest performance and no improvement in crude protein digestibility among the dietary treatments, which indicates the upregulation of nutrient transporters as a means to

compensate the reduction on performance. Kheravii et al. (2018) reported the reduction in weight gain and the upregulation of intestinal cationic aminoacid transporter 1, and peptide transporter-2 in broilers fed 2% sugar bagasse with a coarse particle size. In accordance with these authors, the interaction, fiber type × particle size, showed that coarse particle of CL increased the expression of Pept-1 compared to coarse particle of SH in the current study. However, the upregulation of Pept-1 did not result in an improved crude protein digestibility. On the contrary, low levels of insoluble fiber in a fine particle size improved crude protein digestibility, indicating that these improvements in digestibility might be associated to the endogenous amino acid losses; the reduction in the endogenous amino acid losses by fine CL inclusion might result in a lower need of amino acid uptake by the transporters. On the other hand, the higher abrasive effect of coarse particles increases the need for amino acid uptake, upregulating the expression of Pept-1. Furthermore, birds from the fine CL group had the lowest feed intake which can be attributed to the slower digesta passage rate due to the bulkiness of the diets containing cellulose. Similar to these results, Khempaka et al. (2009) reported a reduction in feed intake with increases in died cassava pulp containing 27% insoluble fiber. Therefore, the reduction in feed intake leads to a lower nutrient intake and a higher need to compensate for the lack of nutrients stimulating the upregulation of nutrient transporters such as Pept-1.

Conclusions

Dietary fiber type, inclusion level, and particle size are important factors determining the functionality of the fibrous feed components. In the present study, it was observed that 4% SH diets with fine particles had similar weigh gain compared to the control group and improved the feed efficiency during the first week of the experiment. An improvement in jejunal villus to crypt ratio was also observed in such diets, indicating a positive modulation of the gastrointestinal

tract. High levels of SH increased relative weights of the gizzard and small intestine but reduced performance and increase mortality due to increases in intestinal viscosity. This indicates that SH can be added to diets to achieve 4% CF (i.e. 5-6% in a corn-soybean meal diet) without causing any deleterious effect and with a high potential to improve intestinal functionality. Expression of Pept-1was not related to digestibility of crude protein due to potential endogenous loses caused by abrasion of dietary fiber. In summary, type of fiber, inclusion level, and particle size should be considered when using fibrous feedstuffs since these are determining factors affecting growth performance, intestinal morphology, nutrient digestibility, nutrient metabolism, and further research is granted to understand the role of fibrous feed ingredient in the nutrient matrix. This will provide us a way to be able to incorporate cheaper feed ingredients.

References

Amerah, A. M., V. Ravindran, and R. G. Lentle. 2009. Influence of insoluble fibre and whole wheat inclusion on the performance, digestive tract development and ileal microbiota profile of broiler chickens. Br. Poult. Sci., 50:366–375.

Amerah, A. M., V. Ravindran, R. G. Lentle, and D. G. Thomas. 2007. Feed particle size: Implications on the digestion and performance of poultry. W. Poult. Sci. J. 63:439–455.

Cao, B. H., X. P. Zhang, Y. M. Guo, Y. Karasawa, and T. Kumao. 2003. Effects of dietary cellulose levels on growth, nitrogen utilization, retention time of diets in digestive tract and caecal microflora of chickens. Asian-Australasian J. Anim Sci., 16: 863–866.

Carré, B., N. Muley, J. Gomez, F. X. Oury, E. Laffitte, D. Guillou, and C. Signoret. 2005. Soft wheat instead of hard wheat in pelleted diets results in high starch digestibility in broiler

chickens. Br. Poult. Sci. 46:66–74.

Chaplin, M. F. 2003. Fibre and water binding. Proc. Nutr. Soc. 62:223–227.

Chiou, P. W. S., T. W. Lu, J. C. Hsu, and B. Yu. 1996. Effect of different sources of fiber on the intestinal morphology of domestic geese. Asian-Australasian J. Anim. Sci. 9:539–550.

Choct, M. (2015). Fibre - Chemistry and Functions in Poultry Nutrition. LII Simp. Cient. de Avicultura, 113–119.

Cobb-vantress. 2018. Broiler Management Guide. Accessed Jan. 2021. https://www.cobb-vantress.com/products/cobb500.

Cobb-Vantress. 2018. Broiler Performance and Nutrition Supplement Cobb500. Accessed Jan. 2021. http://www.cobb-vantress.com/docs/default-source/cobb-500-guides/cobb500-broiler-performance-nutrition-supplement-(english).pdf

Dansky, L. M., and F. W. Hill. 1952. Application of chromic oxide indicator method to balance studies with growing chickens: two figures. J. Nutr. 47:449-459.

Donadelli, R. A., D. A. Stone, C. G. Aldrich, and R. S. Beyer. 2019. Effect of fiber source and particle size on chick performance and nutrient utilization. Poult. Sci. 98:5820–5830.

Festucci-Buselli, R. A., W. C. Otoni, and C. P. Joshi. 2007. Structure, organization, and functions of cellulose synthase complexes in higher plants. Braz. J. Plant Physiol. 19:1–13.

Georgieva, V., S. Chobanova, N. Todorov, and D. Pavlov. 2014. Effect of dietary crude fiber on endogenous dry matter and nitrogen excretion in cockerels. Bul. J. Ag. Sci.20:903–908.

Gonzalez-Alvarado, J. M., E. Jimenez-Moreno, R. Lazaro, G. Mateos. 2007. Effect of type of cereal, heat processing of the cereal, and inclusion of fiber in the diet on productive performance and digestive traits of broilers. Poult. Sci., 86:1705–1715.

González-Alvarado, J. M., E. Jiménez-Moreno, D. G. Valencia, R. Lázaro, G. Mateos. 2008. Effects of fiber source and heat processing of the cereal on the development and pH of the gastrointestinal tract of broilers fed diets based on corn or rice. Poult. Sci. 87:1779–1795.

Hetland, H., M. Choct, and B. Svihus. 2004. Role of insoluble non-starch polysaccharides in poultry nutrition. W. Poult. Sci. J. 60:415–422.

Hetland, H., and B. Svihus. 2001. Effect of oat hulls on performance, gut capacity and feed passage time in broiler chickens. Br. Poult. Sci. 42:633–637.

Hetland, H., B. Svihus, and A. Krogdahl. 2003. Effects of oat hulls and wood shavings on digestion in broilers and layers fed diets based on whole or ground wheat. Br. Poult. Sci. 44: 275–282.

Hivechi, A., and S. H. Bahrami. 2016. A new cellulose purification approach for higher degree of polymerization: Modeling, optimization and characterization. Carb. Pol. 152:280–286.

Jaworski, N. W., H. N. Lærke, K. E. Knudsen, and H. H. Stein. 2015. Carbohydrate composition and in vitro digestibility of dry matter and nonstarch polysaccharides in corn, sorghum, and wheat and coproducts from these grains. J. Anim. Sci. 93:1103–1113.

Jiménez-Moreno, E., A. De Coca-Sinova, J. M. González-Alvarado, G. Mateos. 2016. Inclusion of insoluble fiber sources in mash or pellet diets for young broilers. 1. Effects on growth

performance and water intake. Poult. Sci. 95:41–52.

Khempaka, S., W. Molee, and M. Guillaume. 2009. Dried cassava pulp as an alternative feedstuff for broilers: Effect on growth performance, carcass traits, digestive organs, and nutrient digestibility. J. Appl. Poult. Res. 18:487–493.

Kheravii, S. K., N. K. Morgan, R. A. Swick, M. Choct, and S. B. Wu. 2018. Roles of dietary fibre and ingredient particle size in broiler nutrition. W. Poult. Sci. J. 74:301–316.

Kheravii, S., R. Swick, M. Choct, S. Wu. 2018. Upregulation of genes encoding digestive enzymes and nutrient transporters in the digestive system of broiler chickens by dietary supplementation of fiber and inclusion of coarse particle size corn. BMC Genom. 19:1–14.

Knudsen, K. E. B. (2014). Fiber and nonstarch polysaccharide content and variation in common crops used in broiler diets. Poult. Sci. 93:2380–2393.

Mitchell, R. J., P. W. Waldroup, C. M. Hillard, and K. R. Hazen. 1972. Effects of Pelleting and Particle Size on Utilization of Roasted Soybeans by Broilers. Poult. Sci. 51:506–510.

Montagne, L., J. R. Pluske, D. J. Hampson. 2003. A review of interactions between dietary fibre and the intestinal mucosa, and their consequences on digestive health in young non-ruminant animals. Anim. F. Sci. Technol. 108:95–117.

Nguyen, N., M. Jacobs, J. Li, C. Huang, D. Li, D. L. Navarro, H. H. Stein, N. W. Jaworski. 2019. Technical note: Concentrations of soluble, insoluble, and total dietary fiber in feed ingredients determined using method AOAC 991.43 are not different from values determined using method AOAC 2011.43 with the AnkomTDF dietary fiber analyzer. J.

Anim. Sci. 97:3972–3983.

O'Dell, B. L., P. M. Newberne, and J. E. Savage. 1959. An Abnormality of the proventriculus caused by feed texture. Poult. Sci. 38:296–301.

Owusu-Asiedu, A., J. F. Patience, B. Laarveld, A. G. Van Kessel, P. H. Simmins, and R. T. Zijlstra. 2006. Effects of guar gum and cellulose on digesta passage rate, ileal microbial populations, energy and protein digestibility, and performance of grower pigs. J. Anim. Sci. 84:843–852.

Pan, D., and Z. Yu. 2014. Intestinal microbiome of poultry and its interaction with host and diet. 5:108–119.

Rezaei, M., M. A. Karimi Torshizi, H. Wall, and E. Ivarsson. 2018. Body growth, intestinal morphology and microflora of quail on diets supplemented with micronised wheat fibre. Br. Poult. Sci. 59:422–429.

Sacranie, A., B. Svihus, V. Denstadli, B. Moen, P. A. Iji, and M. Choct. 2012. The effect of insoluble fiber and intermittent feeding on gizzard development, gut motility, and performance of broiler chickens. Poult. Sci. 91:693–700.

Sadeghi, A., M. Toghyani, and A. Gheisari. 2015. Effect of various fiber types and choice feeding of fiber on performance, gut development, humoral immunity, and fiber preference in broiler chicks. Poult. Sci. 94:2734–2743.

Saki, A. A., H. R. H. Matin, P. Zamani, M. M.Tabatabai, and M. Vatanchian. 2011. Various ratios of pectin to cellulose affect intestinal morphology, DNA quantitation, and

performance of broiler chickens. Liv. Sci. 139:237–244.

Silva, V. K., V. de Souza Morita, and I. C. Boleli. 2013. Effect of pectin extracted from citrus pulp on digesta characteristics and nutrient digestibility in broilers chickens. Rev. Bras. Zootec. 42:575–583.

Sittiya, J., K. Yamauchi, W. Nimanong, and N. Thongwittaya. 2020. Influence of levels of dietary fiber sources on the performance, carcass traits, gastrointestinal tract development, fecal ammonia nitrogen, and intestinal morphology of broilers. Rev. Bras. Cien. Avi. 22:1–8.

Sklan, D., A. Smirnov, A., and I. Plavnik. 2003. The effect of dietary fibre on the small intestines and apparent digestion in the turkey. Br. Poult. Sci. 44:735–740.

Stein, H. H., and C. M. Parsons. 2008. Nutritional properties and feeding values of soybeans and their coproducts. Pages 613-660 in Soybeans, Chemistry, production, processing, and utilization. L. A. Johnson, P. J. White and R. Galloway, eds. AOCS Press, Urbana IL.

Strange, E. D., and C. I. Onwulata. 2002. Effect of particle size on the water sorption properties of cereal fibers. J. F. Qual. 25:63–73.

Svihus, B. 2011. The gizzard: Function, influence of diet structure and effects on nutrient availability. W. Poult. Sci. J. 67:207–223.

Tejeda, O. J., and W. K. Kim. 2020. The effects of cellulose and soybean hulls as sources of dietary fiber on the growth performance, organ growth, gut histomorphology, and nutrient digestibility of broiler chickens. Poult. Sci. 99:6828–6836.

Tejeda, O. J., and W. K. Kim. 2021. Role of dietary fiber in poultry nutrition. Anim. 11:1–16.

Welch, R. W., M. V. Hayward, and D. I. H. Jones. 1983. The composition of oat husk and its variation due to genetic and other factors. J. Sci. F. Agr. 34:417–426.

Table 5.1. Proximate analyses of the nutrient composition of soybean hulls

Item	Value
Gross energy (Kcal/Kg)	3,698
N-corrected apparent metabolizable energy (Kcal/kg)	658
Dry Matter (%)	87.95
Crude Protein (%)	16.3
Crude Fiber (%)	35.8
Calcium (%)	0.88
Phosphorus (%)	0.55
Non-phytate phosphorus (%)	0.37

Table 5.2. Analyzed values for amino acid content, digestibility (%) and digestible amino-acid content of Soybean Hulls based on cecectomized rooster assay.

Amino Acid	Percent Amino acid	Digestibility (%)	Digestible Amino acid content (%)
Alanine	0.450	39.386	0.177
Arginine	0.480	68.344	0.328
Aspartic acid	0.950	54.040	0.513
Cysteine	0.160	41.461	0.066
Glutamic acid	1.140	51.294	0.585
Histidine	0.260	46.506	0.121
Isoleucine	0.400	43.850	0.175
Leucine	0.660	52.328	0.345
Lysine	0.730	52.738	0.383
Methionine	0.130	57.583	0.075
Phenylalanine	0.390	53.601	0.209
Proline	0.550	55.592	0.306
Serine	0.530	49.335	0.261
Threonine	0.360	50.722	0.183
Tryptophan	0.060	56.100	0.034
Tyrosine	0.360	46.732	0.168
Valine	0.460	33.951	0.156

Table 5.3. Ingredient composition of diets fed to male Cobb × Cobb broilers from 1 to 21 days of age[1]

Ingredient, %	CTL	4% CL	8% CL	4% SH	8% SH
Corn	49.56	49.56	49.56	53.59	38.14
Soybean Meal	35.09	35.09	35.09	32.37	30.48
Solka floc®	---	2.03	6.07	---	---
Soybean hulls	---	---	---	5.62	17.77
Soybean oil	5.01	5.01	5.01	3.76	9.24
Defluorinated Phosphate	1.05	1.05	1.05	0.168	0.63
Biofos 16/21P	0.55	0.55	0.55	1.29	0.98
Calcium carbonate	0.91	0.91	0.91	1.24	0.72
L-Thr	0.12	0.12	0.12	0.15	0.20
DL-Met	0.32	0.32	0.32	0.33	0.36
Lysine HCl	0.20	0.20	0.20	0.26	0.31
Vitamin premix[2]	0.25	0.25	0.25	0.25	0.25
Mineral premix[3]	0.15	0.15	0.15	0.15	0.15
Sodium Chloride	0.23	0.23	0.23	0.33	0.28
Filler (sand)	6.56	4.74	0.5	0.5	0.50
Dry matter (%)	90	90	90	90	90
Calculated Nutrient Composition					
ME energy (Kcal/kg)	3,000	3,000	3,000	3,000	3,000
Protein (%)	21.0	21.0	21.0	21.0	21.0
Crude Fiber (%)	2.0	4.0	8.0	4.0	8.0
Calcium (%)	0.90	0.90	0.90	0.90	0.90
Dig. Phosphorus (%)	0.45	0.45	0.45	0.45	0.45
Dig. Met (%)	0.63	0.63	0.63	0.63	0.63
Dig. TSAA (%)	0.90	0.90	0.90	0.90	0.90
Dig. Lys (%)	1.22	1.22	1.22	1.22	1.22
Dig. Thr (%)	0.86	0.86	0.86	0.86	0.86

[1]All diets, except control, were added fiber at a particle size of 100 and 600 µm.
[2]Vitamin premix provided the following per kilogram of DSM premix: Vit. A, 2,204,586 IU; Vit. D_3, 200,000 ICU; Vit. E, 2,000 IU; Vit. B12, 2 mg; Biotin, 20 mg; Menadione, 200 mg; Thiamine, 400 mg; Riboflavin, 800 mg; d-Pantothenic Acid, 2,000 mg; Vit. B6, 400 mg; Niacin, 8,000 mg; Folic Acid, 100 mg; Choline, 34,720 mg.
[3]Mineral premix includes per kg of premix: Ca, 0.72 g; Mn, 3.04 g; Zn, 2.43 g; Mg, 0.61 g; Fe, 0.59 g; Cu, 22.68 g; I, 22.68 g; Se, 9.07 g.

Table 5.4. Primer pairs used for RT-qPCR analyses

Gene[1]	Gene bank identification	Primer sequence, forward/reverse	Product size (bp)
SGLT-1	AJ236903.1	GCCGTGGCCAGGGCTTA/ CAATAACCTGATCTGTGCACCAGT	71
Pept-1	KF366603.1	CCCCTGAGGAGGATCACTGTT/ CAAAAGAGCAGCAGCAACGA	66
GAPDH	NC_052532.1	GCTAAGGCTGTGGGGAAAGT/ TCAGCAGCAGCCTTCACTAC	161

[1]SGLT-1 = sodium-dependent glucose transporter 1; Pept-1 = peptide transporter-1; GAPDH = glyceraldehyde 3-phosphate dehydrogenase.

Table 5.5 Effects of dietary fiber parameters on the growth performance of male broilers reared to 21 d of age[1]

			BW gain (g/bird)			Feed intake (g/bird)			Feed conversion			Mort., %
Fiber type[2]	**Particle size (µm)**	**Level**	**D 7**	**D 14**	**D 21**	**D 7**	**D 14**	**D 21**	**D 7**	**D 14**	**D 21**	**D0-21**
Control	**-**	**-**	124^{a}	432^{a}	884^{a}	144^{a}	656^{a}	1639^{a}	1.17^{ab}	1.52	1.85	2.78
CL	**100**	**4%**	114^{ab}	396^{ab}	813^{abc}	128^{ab}	527^{bc}	1350^{ab}	1.12^{a}	1.33	1.67	2.78
CL	**100**	**8%**	104^{bc}	324^{cd}	658^{d}	122^{bc}	480^{bc}	1233^{b}	1.18^{ab}	1.48	1.87	4.20
CL	**600**	**4%**	107^{bc}	377^{bc}	818^{abc}	131^{ab}	558^{ab}	1640^{a}	1.23^{b}	1.50	2.00	5.60
CL	**600**	**8%**	85^{d}	279^{d}	641^{d}	105^{c}	429^{c}	1268^{b}	1.24^{b}	1.54	1.98	2.78
SH	**100**	**4%**	112^{abc}	407^{ab}	870^{ab}	127^{ab}	566^{ab}	1497^{ab}	1.14^{a}	1.39	1.72	5.56
SH	**100**	**8%**	96^{cd}	303^{d}	665^{cd}	118^{bc}	473^{bc}	1292^{b}	1.23^{b}	1.56	1.94	4.20
SH	**600**	**4%**	115^{ab}	368^{bc}	767^{abcd}	133^{ab}	524^{bc}	1345^{ab}	1.16^{ab}	1.42	1.75	2.50
SH	**600**	**8%**	97^{cd}	305^{d}	724^{bcd}	119^{bc}	484^{bc}	1427^{ab}	1.23^{b}	1.60	1.98	8.33
Standard Error			3.50	12	34	4.30	27.00	99.00	0.03	0.06	0.10	1.35
P-value			<0.01	<0.01	<0.01	<0.01	<0.01	0.030	0.015	0.090	0.162	0.436
Main effects												
Fiber type	CL		103	344	732	121	498	1373	1.19	1.46	1.88	3.84^{b}
	SH		105	346	757	124	512	1390	1.19	1.49	1.85	5.15^{a}
Particle size (µm)	100		107^{a}	358^{a}	752	124	511	1343	1.16^{a}	1.44	1.80	3.83
	600		101^{b}	332^{b}	737	122	499	1420	1.21^{b}	1.51	1.93	4.80
Level	4%		112^{a}	387^{a}	817^{a}	130^{a}	544^{a}	1458^{a}	1.16^{a}	1.41^{a}	1.78^{a}	4.11
	8%		96^{b}	303^{b}	672^{b}	116^{b}	466^{b}	1305^{b}	1.22^{b}	1.54^{b}	1.94^{b}	4.88
Source of variation (P-value)												
Fiber type (T)			0.340	0.841	0.247	0.297	0.403	0.788	0.969	0.433	0.637	0.002
Particle size (P)			0.021	0.004	0.489	0.529	0.424	0.242	0.019	0.062	0.073	0.980
Inclusion level (L)			<0.01	<0.01	<0.01	<0.01	<0.01	0.023	0.006	0.002	0.028	0.468
T × P			0.002	0.436	0.6916	0.077	0.862	0.197	0.057	0.346	0.209	<0.01
T × P × L			0.340	0.052	0.032	0.181	0.035	0.044	0.680	0.492	0.410	<0.01

[1]Values are the least-square means of 6 replicate cages per treatment, each cage with 12 birds.

[2]CL = cellulose, SH = soyhulls. [a-d]Means within a column not sharing a common superscript differ significantly (P < 0.05).

Table 5.6. Effects of dietary fiber parameters on the intestinal histomorphology of male broilers reared to 21 days of age[1]

			Duodenum			Jejunum			Ileum		
Fiber type[2]	**Particle size (µm)**	**Level**	**Villus (µm)**	**Crypt (µm)**	**Ratio (µm)**	**Villus(µm)**	**Crypt (µm)**	**Ratio (µm)**	**Villus (µm)**	**Crypt (µm)**	**Ratio (µm)**
Control	**-**	**-**	3067^{ab}	269^{a}	11.8^{ab}	1817^{a}	220	8.8^{ab}	1340^{a}	231^{ab}	6.3^{ab}
CL	**100**	**4%**	2848^{bc}	250^{ab}	11.9^{ab}	1650^{abc}	214	8.0^{abcd}	1017^{b}	206^{b}	5.17^{bc}
CL	**100**	**8%**	2643^{c}	262^{ab}	10.5^{b}	1597^{abc}	235	7.1^{bcd}	1029^{b}	202^{b}	5.27^{bc}
CL	**600**	**4%**	2841^{bc}	236^{b}	13.0^{a}	1453^{cd}	217	7.2^{bcd}	1045^{b}	204^{b}	5.3^{bc}
CL	**600**	**8%**	2949^{ab}	243^{ab}	12.6^{a}	1644^{abc}	231	7.3^{abcd}	1058^{b}	211^{ab}	6.69^{a}
SH	**100**	**4%**	2962^{ab}	256^{ab}	12.3^{ab}	1758^{ab}	203	9.0^{a}	1004^{b}	208^{ab}	5.03^{c}
SH	**100**	**8%**	3079^{ab}	241^{ab}	13.3^{a}	1237^{d}	215	6.2^{d}	1023^{b}	210^{ab}	5.04^{c}
SH	**600**	**4%**	3163^{a}	248^{ab}	13.1^{a}	1654^{abc}	224	8.0^{abcd}	1138^{b}	243^{a}	4.96^{c}
SH	**600**	**8%**	2900^{abc}	247^{ab}	12.2^{ab}	1493^{bcd}	232	6.9^{cd}	1041^{b}	244^{a}	4.42^{c}
Standard Error			64.00	8.00	0.50	71	10	0.4	45	11	0.5
P-value			<0.01	0.030	<0.01	<0.01	0.202	<0.01	<0.01	<0.01	0.031
Main effects											
Fiber type	CL		2682	238	11.79	1352	229^{b}	6.25	977^{a}	208	4.97^{a}
	SH		2731	231	12.29	1369	248^{a}	5.98	857^{b}	197	4.63^{b}
Particle size (µm)	100		2619^{b}	227^{b}	11.96	1323^{b}	228^{b}	6.26	907	197	4.87
	600		2793^{a}	242^{a}	12.11	1398^{a}	249^{a}	5.97	927	207	4.73
Level	4%		2852^{a}	240^{a}	12.4^{a}	1411^{a}	247^{a}	6.04^{b}	868^{b}	204	4.49^{b}
	8%		2561^{b}	229^{b}	11.67^{b}	1310^{b}	230^{b}	6.19^{a}	965^{a}	201	5.11^{a}
Source of variation (P-value)											
Fiber type (T)			0.077	0.188	0.106	0.497	0.003	0.132	<0.001	0.0607	0.0421
Particle size (P)			<0.001	0.005	0.632	0.002	0.001	0.098	0.3644	0.0955	0.3982
Inclusion level (L)			<0.001	0.038	0.018	<0.001	0.010	0.387	<0.001	0.637	<0.001
T × P			0.030	0.417	0.199	0.078	0.238	0.922	0.1958	0.1931	0.497
T × P × L			0.206	0.441	0.122	0.146	0.002	<0.001	0.7287	0.5506	0.6563

[1]Values are the least-square means of 6 replicate birds per treatment.
[2]CL = cellulose, SH = soyhulls. [a-d]Means within a column not sharing a common superscript differ significantly ($P < 0.05$).

Table 5.7. Effects of dietary fiber parameters on the relative organ weights of male broilers reared to 21 days of age[1]

			Gizzard, %		Small Intestine, %		Ceca, %	
Fiber type[2]	**Particle size (µm)**	**Level**	**Day 14**	**Day 21**	**Day 14**	**Day 21**	**Day 14**	**Day 21**
Control	**-**	**-**	3.6^{ab}	2.48^{ab}	9.86	6.31^{b}	0.93	0.74
CL	**100**	**4%**	3.15^{b}	2.48^{ab}	10.63	7.95^{ab}	1.09	1.02
CL	**100**	**8%**	3.30^{b}	2.53^{ab}	10.96	7.76^{ab}	1.17	0.9
CL	**600**	**4%**	3.73^{ab}	2.53^{ab}	10.96	8.94^{ab}	1.03	1.04
CL	**600**	**8%**	3.93^{ab}	2.93^{ab}	9.98	9.25^{ab}	1.41	1.18
SH	**100**	**4%**	3.79^{ab}	2.41^{b}	9.71	7.88^{ab}	1.14	0.97
SH	**100**	**8%**	4.17^{a}	2.63^{ab}	11.65	10.19^{a}	1.09	0.82
SH	**600**	**4%**	3.45^{ab}	2.60^{ab}	10.57	7.95^{ab}	1.08	0.88
SH	**600**	**8%**	4.09^{a}	3.27^{a}	11.52	9.82^{a}	1.19	0.95
Standard Error			0.17	0.19	0.69	0.70	0.13	0.13
P-value			<0.001	0.045	0.419	0.009	0.413	0.408
Main effects								
Fiber type	CL		3.53^{b}	2.62	10.63	8.48	1.17	1.04
	SH		3.87^{a}	2.73	10.86	8.96	1.12	0.90
Particle size (µm)	100		3.60	2.51^{b}	10.74	8.44	1.12	0.93
	600		3.80	2.83^{a}	10.76	8.99	1.17	1.01
Level	4%		3.53^{b}	2.5^{b}	10.47	8.18^{b}	1.08	0.96
	8%		3.87^{a}	2.84^{a}	11.03	9.26^{a}	1.21	0.98
Source of variation (P-value)								
Fiber type (T)			0.006	0.437	0.634	0.338	0.569	0.144
Particle size (P)			0.102	0.026	0.969	0.276	0.554	0.348
Inclusion level (L)			0.007	0.020	0.250	0.037	0.162	0.883
Type × Particle size			0.002	0.500	0.476	0.171	0.719	0.449
Type × Level			0.163	0.436	0.073	0.047	0.285	0.765

[1]Values are the least-square means of 6 replicate birds per treatment. [2]CL = cellulose, SH = soyhulls.

Table 5.8. Effects of dietary fiber parameters on the intestinal viscosity of male broilers reared to 21 days of age[1]

			Viscosity, mPas		
Fiber type[2]	**Particle size (µm)**	**Level**	**Day 7**	**Day 14**	**Day 21**
Control	**-**	**-**	6.65^{ab}	2.26^{b}	2.25^{c}
CL	**100**	**4%**	9.07^{ab}	2.36^{b}	2.02^{c}
CL	**100**	**8%**	3.63^{bc}	2.19^{b}	2.39^{c}
CL	**600**	**4%**	7.47^{ab}	3.35^{b}	3.03^{bc}
CL	**600**	**8%**	1.78^{c}	2.13^{b}	2.41^{bc}
SH	**100**	**4%**	7.85a^{b}	3.45^{b}	2.40^{c}
SH	**100**	**8%**	11.38^{ab}	6.44^{a}	7.75^{a}
SH	**600**	**4%**	6.08^{ab}	3.06^{b}	3.4b^{c}
SH	**600**	**8%**	13.23^{a}	7.38^{a}	5.74^{ab}
Standard Error			2.80	0.86	0.083
P-value			0.045	<0.001	<0.001
Main effects					
Fiber type	CL		5.58^{b}	2.54^{b}	2.43^{b}
	SH		9.79^{a}	4.87^{a}	4.55^{a}
Particle size (µm)	100		7.93	3.67	3.23
	600		7.71	3.94	3.8
Level	4%		7.63	3.04^{b}	2.75^{b}
	8%		8.02	4.74^{a}	4.45^{a}
Source of variation (P-value)					
Fiber type (T)			0.020	<0.001	<0.001
Particle size (P)			0.622	0.474	0.993
Inclusion level (L)			0.948	0.006	0.001
Type × Level			0.003	<0.001	<0.001

[1]Values are the least-square means of 6 replicate birds per treatment.
[2]CL = cellulose, SH = soyhulls. [a-d]Means within a column not sharing a common superscript differ significantly ($P < 0.05$).

Table 5.9. Effects of dietary fiber parameters on the nutrient digestibility of male broilers reared to 21 days of age[1]

Fiber type[2]	Particle size (µm)	Level	Dry matter, %	Crude protein, %	Energy, kcal/kg
Control	**-**	**-**	63.83^{ab}	82.30^{abcd}	3057
CL	**100**	**4%**	69.85^{ab}	86.59^{a}	3006
CL	**100**	**8%**	67.44^{ab}	84.38^{abc}	2842
CL	**600**	**4%**	68.20^{ab}	83.61^{abcd}	2811
CL	**600**	**8%**	61.23^{ab}	80.75^{bcd}	2641
SH	**100**	**4%**	71.24^{a}	85.11^{ab}	2938
SH	**100**	**8%**	64.19^{ab}	84.42^{abc}	2797
SH	**600**	**4%**	62.89^{ab}	79.38^{d}	2720
SH	**600**	**8%**	59.02^{b}	79.58^{cd}	2589
Standard Error			2.72	1.80	170
P-value			0.0169	0.0326	0.0730
Main effects					
Fiber type	CL		66.68	83.83	2825
	SH		64.33	82.12	2761
Particle size (µm)	100		68.18^{a}	85.12^{a}	2896^{a}
	600		62.84^{b}	80.83^{b}	2690^{b}
Level	4%		68.04^{a}	83.67	2869
	8%		62.97^{b}	82.28	2717
Source of variation (P-value)					
Fiber type (T)			0.2008	0.1577	0.4001
Particle size (P)			0.0052	0.0008	0.0097
Inclusion level (L)			0.0077	0.2484	0.0515
Type × Particle size			0.4373	0.4105	0.9230

[1]Values are the least-square means of 6 replicate cages per treatment, each cage with 12 birds.
[2]CL = cellulose, SH = soyhulls. [a-d]Means within a column not sharing a common superscript differ significantly ($P < 0.05$).

Table 5.10. Effects of dietary fiber parameters on the jejunal gene expression of male broilers reared to 21 d of age[1]

			Gene	
Fiber type[2]	**Particle size (µm)**	**Level**	**SGLT-1**	**Pept-1**
Control	**-**	**-**	1.00	1.00[b]
CL	**100**	**4%**	0.447	3.17[ab]
CL	**100**	**8%**	0.245	1.56[ab]
CL	**600**	**4%**	0.294	2.63[ab]
CL	**600**	**8%**	1.244	4.11[a]
SH	**100**	**4%**	0.442	3.27[ab]
SH	**100**	**8%**	0.075	2.39[ab]
SH	**600**	**4%**	0.237	1.19[ab]
SH	**600**	**8%**	0.085	1.6a[b]
Standard Error			0.344	0.700
P-value			0.080	<0.010
Main effects				
Fiber type	CL		0.558	2.87
	SH		0.210	2.11
Particle size (µm)	100		0.302	2.38
	600		0.465	2.60
Level	4%		0.355	2.56
	8%		0.412	2.41
Source of variation (P-value)				
Fiber type (T)			0.122	0.124
Particle size (P)			0.463	0.656
Inclusion level (L)			0.795	0.752
Type × Particle size			0.244	0.015

[1]Values are the least-square means of 6 replicate birds per treatment.
[2]CL = cellulose, SH = soyhulls. [a-d]Means within a column not sharing a common superscript differ significantly ($P < 0.05$).

CHAPTER 6

EFFECTS OF SOYHULLS-ADDED 44% CRUDE PROTEIN SOYBEAN MEAL AND EXOGENOUS BETA-MANNANASE ON THE GROWTH PERFORMANCE, INTESTINAL MORPHOLOGY, NUTRIENT DIGESTIBILITY, GENE EXPRESSION, AND VOLATILE FATTY ACIDS OF BROILERS[1]

[1]Tejeda, O. J., A. Sing, T. J. Applegate, C. W. Ritz, H. Thippareddi, T. Callaway, J. M. Lourenco, and W. K. Kim. Submitted to Poultry Science.

Abstract

The aim of this study was to evaluate the effect soybean meal type and β-mannanase inclusion level on the growth performance, intestinal morphology, gene expression, nutrient digestibility, and volatile fatty acid production of broiler chickens. A total of 1,280 one-day-old Cobb male broilers were allocated in a completely randomized block design with 8 dietary treatments organized as a 4 (enzyme levels) × 2 (soybean meal types: 48% vs. 44% CP) factorial design with 8 replicates each. Birds were reared to 42 days and provided with diets divided in starter, grower and finisher phases. Two basal diets (one per SBM type) were formulated and mixed in each feeding phase to which different levels of β-mannanase were added namely none (0 IU), 0.5 × recommended (21,750 IU), 1 × recommended (43.500 IU), and 2 × recommended (87,000 IU) manufacturer's level. All diets were formulated to be isonitrogenous and isocaloric. Chromium oxide was added to all diets on day 37 for analyses of nutrient digestibility. Growth performance was measured on days 14, 28, and 42. Intestinal samples from the duodenum, jejunum, and ileum were collected on days 14 and 35 for analyses of intestinal morphology. On days 14 and 35 samples from the jejunal mucosa, spleen, and cecal tonsils were collected for analyses of tight junction and immune genes. On day 35, cecal and ileal contents were collected for analyses of volatile fatty acids. On day 42, ilea digesta from 7 birds per pen was collected for analyses of nutrient digestibility. Additions of 87,000 IU (2×) of β-mannanase reduced the weight gains ($P < 0.01$). The feed efficiency was improved in the group fed 44% SBM without the inclusion of the enzyme on days 14 and 35 ($P = 0.008$). No significant differences were observed in the feed intake among dietary treatments ($P > 0.05$). The presence of β-mannanase decreased jejunal villus, villus to crypt ratio, and ileal villus height ($P < 0.01$). Expression of splenic IL-10 and cecal tonsil IL-1b was upregulated in the groups 44% SBM with 1× enzyme, and 44% SBM

without enzyme, respectively ($P < 0.05$). The presence of β-mannanase improved the digestibility of dry matter, energy, acid detergent fiber, and crude fat ($P < 0.01$). However, when added at 2 × in the groups containing 44% SBM, resulted in impaired nutrient digestibility ($P < 0.01$). No differences in the production of volatile fatty acids was observed among dietary treatments ($P > 0.05$). In conclusion, 44% SBM can modulate the intestinal morphology, gene expression and the addition of exogenous β-mannanase does not seem to improve performance parameters when broiler are reared to 42 days.

Key words: β-mannanase, 44% soybean meal, 48% soybean meal, gene expression, nutrient digestibility.

Introduction

Soybean meal (SBM) has become the leading protein source in broiler (and poultry) production providing around 70% of the protein in a typical broiler diet (Waldrup 2006). The poultry industry is the major consumer of soybean meal in the United States, consuming approximately 55% of all U.S. soybean meal (American Soybean Society, 2019). SBM is found in two forms, the typical 48% crude protein (CP) dehulled SBM and the 44% CP SBM with soyhulls (Johnson and Smith, 2004). Soybean hulls (SH) are the by-product of the extraction of soybean oil and make up to 8% of the weight of the grain (Johnson and Smith, 2004). It is estimated that about 5.76 million MT of soyhulls are produced in the US per year (American Soybean Society, 2017); however, most of this by-product goes unused due to their high fiber content and the unwillingness of nutritionists to include fibrous feedstuffs in the nutrient matrix of monogastric animals. This is because several reports have indicated the potential of dietary fiber to negatively affect growth performance (Sadeghi et al., 2015), nutrient digestibility

(Jorgensen et al.,1996), and intestinal health (Jha et al., 2019). However, there is also promising evidence suggesting that dietary fiber can improve growth performance (Adibmoradi et al., 2016; Hetland et al., 2003; Jiménez-Moreno et al., 2009), nutrient digestibility (Amerah et al., 2008; Cao et al., 2003; González-Alvarado et al.,2008), and intestinal morphology (Sklan et al., 2003; Tejeda and Kim, 2020) when used at adequate amounts in diets.

Small additions of soyhulls (6%) has been reported to improve body weight, intestinal development, and nutrient digestibility in mash diets (Sittiya et al., 2020; Tejeda and Kim, 2020). SH are composed of 85% complex carbohydrates divided in 30% pectin, 50% hemicelluloses, and 20% cellulose (Stein and Parsons, 2008). The challenges faced when using fibrous ingredients can be mitigated by the addition of exogenous enzymes (Friesen et al., 1992; Viveros et al., 1994). Furthermore, the breakdown of complex carbohydrates contained in soyhulls could generate volatile fatty acids (VFA) with the potential to modulate immune responses (Walugembe et al., 2015). Therefore, additions of dietary fiber and targeting the breakdown of the major components of these non-starch polysaccharides, namely hemicellulose, with an exogenous enzyme, namely β-mannanase, could provide a path through which this by-product could be included at higher levels in poultry diets. However, little is known about the effect of soyhulls-added 44% CP SBM with soyhulls when added exogenous β-mannanase and used in pelleted diets on the performance, nutrient digestibility, and gene expression of tight junction proteins and immune markers in broilers. Therefore, the objective of this experiment was to determine the effect of 44% CP SBM with soyhulls and different β-mannanase inclusions on the growth performance, nutrient digestibility, intestinal morphology, and gene expression of tight junction proteins and immune parameters in broilers reared to 42 days.

Materials and methods

General procedures

The experiment was approved by the Institutional Animal Care and Use Committee (IACUC) of the University of Georgia (Athens, Georgia, United States). A total of 1,280 one-day-old male Cobb500 broiler chicks were allocated in a 2 × 4 randomized complete block design with 8 dietary treatments and 8 replicates of 20 birds each. The chicks were allocated in 64 floor pens equipped with nipple drinkers and one feeder, providing *ad-libitum* access to water and feed from 1 to 42 days of age. Feed was provided as crumbles during the first two weeks of age (starter phase) and then as pellets during the rest of the experiment (grower and finisher phases). Temperature and lighting program followed the recommendation of Cobb Broiler Management Guide (Cobb-vantress, 2018).

Dietary Treatments

All diets were formulated with corn and soybean meal to meet the nutrient requirements specified by Cobb500 performance and nutritional guide (Cobb-Vantress, 2018). There were 8 dietary treatments divided in 4 treatments containing 48% CP SBM, and other 4 treatments containing 44% CP SBM as the main source of protein. A basal diet for each SBM type was formulated, mixed, and added with none (0 IU), 0.5 × recommended (21,750 IU), 1 × recommended (43.500 IU), and 2 × recommended (87,000 IU) of β-mannanase. Soyhulls were added to common 48% CP SBM to make the 44% CP SBM. Proximate analyses of soyhulls were conducted to measure the gross nutrient content (**Table 6.1**). The nutrient matrix composition (digestible amino acids and nitrogen-corrected apparent metabolizable energy (AMEn)) used for soyhulls were obtained using cecectomized roosters at the poultry research center at the University of Georgia (**Table 6.2**). Starter diets were fed as crumbles from 1 to 14 d

of age, and grower and finisher diets were provided as pellets from 15-28, and 29-42 d, respectively. Basal diets are shown in Table 6.3. All diets were isonitrogenous and isocaloric. For ileal nutrient digestibility determination, chromic oxide (Cr_2O_3, Sigma Aldrich, St. Louis, MO) was added at 0.3% as an indigestible marker to all diets from day 35 to 42.

Growth Performance

The birds and feed were weighed on days 14, 28, and 42 to determine mortality-corrected body weight gain (**BWG**), mortality-corrected feed intake (**FI**), and mortality-corrected feed conversion ratio (**FCR**). Mortality was recorded twice daily.

Intestinal Morphology

On d 14 and 35, samples from the duodenum, jejunum and ileum (~ 5 cm long) were collected from one bird per replicate pen (n = 8 per treatment). Intestinal digesta were flushed with phosphate-buffered saline (PBS) and intestinal sections were stored in 10% neutral-buffered formalin and left in solution for a minimum period of 48 hours for tissue fixation. During slide preparation, increasing amounts of ethanol were used to dehydrate the tissues, and then the tissues were diaphanized in dimethylbenzene, and fixed in paraffin. Finally, tissue sections with a thickness of 4-µm were set on slides and were stained using Hematoxylin and Eosin (H&E) procedures. Pictures were taken using a light microscope (10x eyepiece and 1.6x magnification; Leica DC500 camera, Leica Mycrosystems Inc., Buffalo Groove, IL). Measurements for villi height and crypt depth were taken using ImageJ software (Image Processing and Analysis in JAVA – ImageJ 1.52r, National Instituted of Health).

Nutrient Digestibility

On d 42, six birds per replicate cage were euthanized, and ileal digesta were collected from two-thirds of the distal ileum (from Meckel's diverticulum to about 1 inch anterior to

ileocecal junction). The digesta samples were lyophilized for analyses of dry matter, energy, Acid detergent fiber (ADF), crude fat, crude protein, and amino acids. The chromium oxide concentration was measured according to Dansky and Hill (1952), and gross energy was evaluated using a bomb calorimeter (IKA Calorimeter C1, IKA Works Inc., Wilmington, NC) at the University of Georgia. The crude protein (N × 6.25) and amino acids were analyzed at the Chemical Laboratories at the University of Missouri-Columbia. The apparent ileal digestibility (AID) of all nutrients was calculated using the following equation:

$$AID, \% = 100\left[1 - \left(\frac{Cr_{feed}}{Cr_{dig}}\right) \times \left(\frac{Nutrient_{dig}}{Nutrient_{feed}}\right)\right]$$

where Cr_{feed} and Cr_{dig} are the chromium dioxide in feed and ileal digesta, respectively; and $nutrient_{dig}$ and $nutrient_{feed}$ are the nutrient in ileal digesta and feed, respectively.

Quantitative Reverse-Transcriptase Polymerase Chain Reaction (qRT-PCR)

On d 14 and 35, samples from the jejunal mucosas, cecal tonsils, and spleen were collected from one randomly selected bird, snap-frozen in liquid nitrogen, and stored at -80°C prior to analysis. Jejunal mucosa samples were used to analyze the expression of tight junction genes, claudin and occludin. Splenic and cecal tonsil tissues were used to analyzed the expression of interferon gamma (**IFGM**), interleukin 1-b (**IL-1b**), and interleukin 10 (**IL-10**) genes using quantitative reverse-transcriptase polymerase chain reaction (**qRT-PCR**). Total RNA was extracted from the samples previously stored at -80°C using QIAzol® Lysis Reagent (Qiagen, Germatown, MD) according to the manufacturer's instruction. After extraction, RNA quantity and purity were determined using Nanodrop 1000 spectrophotometer (Thermo Fisher Scientific, Pittsburgh, PA). The cDNA was synthesized from total RNA and subsequently diluted to 10 ng/µl for qRT-PCR analysis. Glyceraldehyde 3-phosphate dehydrogenase (**GAPDH**) was used as the housekeeping gene. The forward and reverse primers for the genes are shown in Table 6.4. The qRT-PCR was

performed on an Applied Biosystems StepOnePlus™ (Thermo Fisher Scientific, Waltham, MA) with iTaq™ Universal SYBR Green Supermix (BioRad, Hercules, CA). Samples were run in duplicate and relative gene expression data were analyzed using the $2^{-\Delta\Delta Ct}$ method (Livak and Schmittgen, 2001). The mean ΔCt of control group was used to calculate the ΔΔCt value.

Volatile fatty acids

On day 35, cecal and ileal contents were collected and snap-frozen in liquid nitrogen and stored at -80 ºC for further analyzes. Since the samples were in solid state, they were diluted and homogenized following a procedure previously described (Lourenco et al., 2020). Briefly, 1 gram of cecal/ileal content was diluted with 3 grams of DI water, fully homogenized for 1 minute, and subsequently frozen at -20 ºC. The samples were then thawed, centrifuged at 10,000 × *g* for 10 minutes, and 900 µL of supernatant were collected and mixed with 180 µL of fresh 25% (wt/vol) meta-phosphoric acid solution, and immediately frozen at -20 ºC overnight. After at least 24 hours at -20 ºC, samples were thawed, centrifuged at 10,000 × *g* for 10 minutes, and the supernatant was collected and mixed with ethyl acetate in a ratio 1:2 (1-part supernatant to 2 parts of ethyl acetate) and let to settle for 5 minutes. The top layer formed was then transferred to screw-thread vials and analyzed in a gas chromatograph (Shimadzu GC-2010 plus; Shimadzu Corporation, Kyoto, Japan) equipped with an autoinjector (AOC-20i; Shimadzu Corporation, Kyoto, Japan). A capillary column (Zebron ZB-FFAP; 30 m x 0.32 mm x 0.25 µm; Phenomenex Inc., Torrance, CA, USA) was used for separation of the volatile fatty acids. The sample injection volume was set at 1 µL, and the column temperature was initially set at 110 ºC, and gradually increased to 200 ºC over the course of 6 minutes. Helium was used as the carrier gas.

Statistical analyses

Soybean meal type and enzyme inclusion level were the fixed effects in the model. Pen was used as the experimental unit for growth performance and nutrient digestibility; bird was used as the experimental unit for intestinal morphology, VFA, and gene expression. Data were analyzed as a completely randomized block design with 8 treatments organized as 2 × 4 factorial. The main effects model used for statistical analyses is as follows:

$$Y_{ij} = \mu + \alpha_i + \beta_j + \alpha\delta_{ij} + \varepsilon_{ij}$$

where Y_{ij} represents the value for each random variable; μ is the overall mean; α_i, β_j, and $\alpha\beta_{ij}$ are the SBM type and enzyme inclusion level and their interactions, respectively such that $\Sigma\alpha_i=0$; and the random errors ε_{ijt} are identically and independently normally distributed with a mean 0 and a variance σ. All statistical procedures were performed using JMP® Pro (SAS Institute, 2021). In case of significant differences, means were separated using the Tukey's test HSD option. For all hypothesis tests, statistical significance was considered at $P < 0.05$.

RESULTS

Growth performance

The results for growth performance are presented in Table 6.5. The top portion of the table shows the results from the ANOVA analyses and the bottom portion of the table shows the results from the main effects and their interactions. On day 14, the groups fed 44% SBM with the addition of 0, 0.5, or 1× enzyme had a similar weight gain compared to the control group; however, groups fed the highest level of enzyme (2×) had the poorest weight gain regardless of the SBM type ($P < 0.001$). At 28 and 42 days of age, the addition of 87,000 IU (2×) of enzyme reduced the weight gain of the groups fed 48% SBM compared to the group fed 48% SBM with 1× enzyme added and the group 44% SBM without enzyme addition ($P < 0.01$). No significant

differences were observed in the feed intake among dietary treatments during the length of the experiment ($P > 0.05$). The feed efficiency was improved in the group fed 44% SBM without the inclusion of the enzyme on days 14 and 28 ($P = 0.008$). However, such differences disappeared at the end of the experimental period ($P > 0.05$). Results from the main effects show that SBM type did not affect weight gain, feed intake, or feed efficiency significantly ($P > 0.05$). The inclusion 2× of exogenous β-mannanase reduced weight gain during the entire rearing period and feed efficiency on days 14 and 35 ($P < 0.01$). No differences were observed in feed intake due to the addition of the enzyme ($P > 0.05$). The interaction *SBM type* × *enzyme* indicates that the groups 48% SBM with 0.5 × enzyme inclusion and 44% SBM without enzyme inclusion, had higher weigh gain compared to the group fed 48% SBM with 2 × enzyme inclusion ($P = 0.003$).

Intestinal morphology.

Results for intestinal morphology for days 14 and 35 are presented in Tables 6.6 and 6.7, respectively. On day 14, duodenal villus height was higher for the 44 SBM group without enzyme inclusion ($P < 0.01$). The group fed 44% SBM with 1× had the deepest duodenal crypt among dietary treatments ($P < 0.01$). However, there were no significant differences in the duodenal villus/crypt ratio ($P = 0.054$). Jejunal villus height was the highest for the groups fed 48% SBM with 0.5× enzyme inclusion and the lowest for the groups fed 44% SBM with 1 × enzyme inclusion ($P < 0.01$). The deepest jejunal crypt depth was observed in the groups fed 44% SBM with 0.5 × enzyme inclusion among dietary treatments ($P < 0.01$). The highest jejunal villus/crypt ratio was observed in the group fed 48% SBM with 0.5 × enzyme, not differing from the groups fed 0 ×, 0.5 ×, or those fed 44% with no enzyme ($P < 0.01$). The highest ileal villus height was observed in the group fed 48% SBM with 1× enzyme among dietary treatments ($P < 0.01$). Ileal crypt depth was higher in the group fed 48% SBM with 1 × enzyme compared to the

group fed 2 × enzyme. Ileal villus/crypt ratio was higher in the group fed 48% SBM with 1 × compared to the counterpart fed 44% SBM ($P < 0.01$).

On day 35, duodenal villus height was higher for the group fed 44% SBM with 1× enzyme compared to the groups fed 48% SBM with 0.5× enzyme or 44% SBM without enzyme ($P = 0.010$). No significant differences in duodenal crypt depth were observed among the dietary treatments ($P > 0.05$). Duodenal villus height to crypt depth ratio was higher for the group fed 44% with 1× enzyme compared to the groups fed 48% SBM with 1× enzyme ($P = 0.036$). Jejunal villus was higher for the treatments without enzyme addition regardless of the SBM type ($P < 0.01$). No significant differences were observed in the jejunal crypt depth among the dietary treatments ($P > 0.05$). The control group had the highest jejunal villus height to crypt depth ratio compared to the rest of dietary treatments, except the group fed 44% SBM without enzyme ($P < 0.01$). The group fed 44% SBM without enzyme had higher ileal villus height compared to the rest of dietary treatments ($P < 0.01$). No statistical differences were observed in the ileal crypt depth among the dietary treatments ($P > 0.05$). However, the highest level of enzyme reduced ileal villus height to crypt depth ratio in the groups fed 48% SBM but increased ileal villus height to crypt depth ratio in the group fed 44% SBM ($P = 0.01$). Results from the main effects showed that 44% SBM significantly increased jejunal crypt depth compared to SBM 48% ($P = 0.043$). The 44% SBM also increased ileal villus height to crypt ratio when compared to SBM 48% ($P < 0.05$). Higher enzyme inclusion improved duodenal villus height but reduced jejunal villus, jejunal villus height to crypt depth ratio, and ileal villus height ($P < 0.05$). Duodenal villus height to crypt depth ratio was highest for the group fed 44% SBM with 0.5× enzyme and lowest for the group fed 48% SBM 0.5× enzyme ($P = 0.048$). Ileal villus height to crypt depth ratio was increased in the group fed 44% SBM without enzyme ($P = 0.036$). Ileal villus height to crypt

depth ratio was highest for the group fed 44% SBM with 2× enzyme and lowest for the group fed 48% SBM with 2× enzyme ($P = 0.010$).

Gene expression and volatile fatty acids

The results for gene expression for days 14 and 35 are presented in Tables 6.8 and 6.9, respectively. On day 14, no significant differences were observed in the jejunal expression of Occludin or Claudin among dietary treatments ($P > 0.05$). The groups fed 44% SBM with 1 × enzyme inclusion had the highest expression of splenic interferon gamma among dietary treatments, except the 44% SBM without enzyme-fed group ($P < 0.01$). No statistical differences were observed in the expression of splenic IL-1β or IL-10 ($P > 0.05$). No significant differences were observed in the cecal tonsil expression of interferon gamma ($P = 0.07$). However, the groups fed 48% SBM with 0.5 and 2 × enzyme had higher expression of IL-1β and IL-10 ($P < 0.05$). On day 35, no statistical differences were observed in the expression of jejunal Occludin, claudin, or peptide transporter-1 (Pept-1) ($P > 0.05$). No differences were observed in the expression of interferon-γ, or interlukin-1β (IL-1b) in the spleen ($P > 0.05$); however, the groups fed 48% SBM with 2× enzyme, and 44% SBM without and with 1× enzyme had higher expression of interlukin-6 (IL-10) compared to the rest of treatments ($P = 0.016$). In the cecal tonsils, no differences were observed in the expression of interferon-γ, or IL-10 ($P > 0.05$); however, IL-1β was upregulated in the groups fed 44% SBM compared to the rest of dietary treatments ($P = 0.002$). Results from the main effects show that higher inclusions of enzyme reduced the expression of IL-1β in the cecal tonsils ($P = 0.031$). The interaction *SBM type* × *enzyme* is significant for spleen IL-10 and cecal tonsil IL-1β, where spleen IL-10 is upregulated in 44% SBM fed groups compared to those fed 48% SBM, and IL-1β is upregulated in 44% SBM with no enzyme ($P < 0.05$).

The results for fatty acid production are reported in Table 6.10. No statistical differences were observed in the production of cecal fatty acids (P > 0.05).

Nutrient digestibility.

The results for digestibility of DM, energy, ADF, and crude fat are presented in Table 6.11. The lowest dry matter digestibility was observed in the group fed 44% SBM with the highest enzyme inclusion ($P < 0.01$). Enzyme addition improved the digestibility of dry matter when fed at 0.5 and 1× in the groups fed 48% SBM ($P < 0.01$). The lowest energy digestibility was observed in the group fed 44% SBM with 2 × enzyme inclusion ($P < 0.01$). No differences were observed in ADF digestibility among dietary treatments ($P = 0.141$). Groups fed 44% SBM with enzyme inclusion had a reduced crude fat digestibility ($P < 0.01$). Results from the main effects showed that the groups without enzymes and the group with the highest (2×) inclusion of the enzyme had the lowest digestibility of all the nutrients, except ADF ($P < 0.05$). However, the addition of the enzyme at 0.5 or 1× improved nutrient digestibility ($P < 0.05$). The interaction *SBM type* × *enzyme* shows that high inclusions of enzyme in 44% SBM-fed treatments decreased the digestibility of dry matter ($P < 0.01$).

Results for essential and non-essential amino acids are presented in Tables 6.12 and 6.13, respectively. The digestibility of crude protein was improved in the group fed 48% SBM with 1× enzyme ($P < 0.01$). Addition of 2 × enzyme improved the digestibility of all essential amino acids in the groups containing 48% SBM and the opposite was observed for the group added 44% SBM ($P < 0.01$). No differences in essential amino acid digestibility due to SBM type or enzyme inclusion were observed (P > 0.05). Results from the main effects indicate that differences are due to the interaction *SBM type* × *enzyme* where the addition of the enzyme has better effects in the 48% SBM-fed groups ($P < 0.01$). Results for non-essential amino acids

indicate that the control group and the group fed 44% SBM with 2 × enzyme had the lowest digestibility of non-essential amino acids among dietary treatments ($P < 0.01$). Results from the main effects show that SBM type did not affect amino acid digestibility ($P > 0.05$). However, enzyme addition improved the digestibility of cysteine, aspartate and glutamate ($P < 0.05$).

Discussion

Growth performance.

The results from the main effects indicate that most of the effects on growth performance were caused by the addition of β-mannanase. Results from the present experiment indicate that additions of 0.5 and 1 × did not improve weight gain or feed efficiency compared to the control group. Similarly, Lee et al., (2005) reported that addition of exogenous β-mannanase to corn soybean meal diets did not improve growth performance of broilers reared to 6 weeks of age. However, results from the present study indicate that 2 × recommended β-mannanase reduced the weight gain during the entire rearing period and the feed efficiency during the first 4 weeks of age. Other authors have reported the improvement in growth performance parameters when β-mannanase is included to diets, especially those with added extra guar mannans (Daskiran et al., 2004; Lee et al., 2005). Therefore, the effectivity of the enzyme seems to be associated to the level of non-starch polysaccharides in the diet (i.e. mannans). The fact that high inclusions of β-mannanase reduced the weight gain and the feed efficiency when given at doubled amounts as the recommended, especially in diets fed 48% SBM is not clear to explain; however, the results from the present experiment showed a numerical decrease of 3% in the feed intake in the groups fed 48% SBM with 2 × enzyme which might help to explain the reduction in weight gain. In one of their studies using white pekin ducks, Park et al. (2019) reported quadratic improvements in weight gain when feeding ducks with 0 to 0.2% exogenous β-mannanase where inclusions of the enzyme

higher than 0.1% resulted in lower weight gain. However, the reason behind the reduction in feed intake when feeding high levels of exogenous β-mannanase in the present experiment is hard to explain. Mekri et al., (2010) reported numerical reductions in body weight and significant reductions in feed intake in broilers fed 500, 700 or 900 g/ton of exogenous β-mannanase. These authors attributed the reduction in feed intake to the ability of the enzyme to release encapsulated nutrients in the plan cell walls. However, the fact that broilers fed 700 and 900 g/ ton of the enzyme had 6% reduction in body weight in their experiment, might indicate that such reductions in intake are due to other factors that are still unknown. Therefore, this study clearly showed that the highest enzyme inclusion level did not have any beneficial effects and that high levels of exogenous enzymes might abolish the beneficial effects of dietary fiber on growth performance. The group fed 48% SBM with 2 × enzyme had also the lowest feed conversion during the first 4 weeks of age. Zou et al., 2006, reported improvements in feed efficiency in broilers fed 0.025 and 0.05% β-mannanase; however, the inclusion of 0.075% resulted in impaired feed efficiency in the same experiment. Results from the main effects indicate a reduction in weight gain of 4% in the groups fed 2 × enzyme at the end of the study, regardless of the SBM type. Feed efficiency was negatively affected for the groups fed 2× enzyme only compared to the other groups during the first 35 days of the experiment. The average bird weight gain of the 44% SBM without enzyme group was improved by 3% compared to the control group and 7% compared to the group fed 48% SBM with 2 × enzyme. The feed efficiency was the best for the group fed 44% SBM without enzyme among dietary treatments during the first 4 weeks of age. The fact that the weight gain and feed efficiency was improved in the group fed 44% SBM without enzyme addition can be attributed to the impact of the extra dietary fiber included in the diets. The SBM containing 44% crude protein was formulated by mixing common 48% SBM with soybean hulls (at 12% in relation to SBM 48% and

at 3% in the diet) to reduce the crude protein level and increase the crude fiber level. The presence of dietary fiber has been shown to improve weight gain (Jiménez-Moreno et al., 2009; Rezaei, Karimi et al., 2018; Tejeda and Kim, 2020) and feed efficiency (Amerah et al., 2008; Gonzalez-Alvarado, Jimenez-Moreno et al., 2007). Therefore, the addition of fiber in the form of soyhulls resulted in improved weight gain and feed conversion.

Intestinal morphology

Intestinal histomorphological characteristics, namely villus height and crypt depth, are used as useful indicators of the potential absorptive capacity of the small intestines where the enterocyte containing structure (i.e. villus) indicates the potential for nutrient absorption and the enterocyte machinery (i.e. crypt depth) indicates the requirements of epithelial cells (Montagne et al., 2003). In the present experiment, on day 14 the duodenal villus height was higher for groups fed 44% SBM without enzyme inclusion which indicates the potential for hulled-SBM to increase gastrointestinal refluxes as observed by other researchers (Rezaei et al., 2018; Hetland et al., 2003). The main effects also indicate a reduction in duodenal villus height, crypt depth and their respective ratio when given 2 × enzyme. Similar to the results from the present experiment, Mehri et al., (2010) reported decreased duodenal villus/crypt ratio when feeding graded levels of β-mannanase. Because of its role on bolus mixing, these changes in duodenal morphology could be associates with changes in enzymatic dynamics during the process of digestion; however, there are no reports regarding the effects of exogenous enzymes in the functionality of endogenous enzymes in poultry species. Jejunal villus was improved by addition of 0.5 × enzyme to the group fed 48% SBM but the group fed 44% SBM with 1 × enzyme had the shortest jejunal villus height. Jejunal villus/crypt ratio was improved also with the addition of 0.5 × enzyme to the 48% SBM group compared to its counterpart. However, higher additions of the enzyme resulted in reductions in

such parameters. Similarly, high additions of the enzyme negatively affected the ileal development during the first 2 weeks of age. Similar to these results, Park et al. (2019) reported that high inclusions of β-mannanase results in impairment of the intestinal histomorphology increasing crypt depth and reducing villus height. These impairments in intestinal morphology can result in lower nutrient digestibility leading to reductions in growth performance as observed in the groups fed 2× β-mannanase.

Results for day 35 show that the inclusion of β-mannanase up to 1 × increased duodenal villus height in the group fed 44% SBM. Similar to these results, (Karimi and Zhandi, 2014) reported an increased in the duodenal villus length and villus width of broilers fed 1g/kg of β-mannanase during 21 days. However, in the present study, the duodenal villus height to crypt depth ratio was higher for the group fed 44% SBM with 0.5× compared to its counterpart fed 48% SBM. The 44% SBM group was added 3% soyhulls which increases the total fiber content of the diets. The presence of more dietary fiber can increase the gastroduodenal reflux, which includes duodenum and proventriculus, increases the abrasion and stimulation of the duodenal morphology (Duke, 1982). The group fed 44% SBM and the control group had the highest jejunal villus height among the dietary treatments. The jejunal villus height to crypt depth ratio was higher for the control group compared to the rest of the dietary treatments, except the 44% SBM-fed group without enzyme addition. This can be associated to the breakdown of dietary fiber in the diets containing exogenous β-mannanase that changes the physical polymerization of the fiber rendering less abrasive in modulating intestinal morphology as observed with other enzymes during *in vitro* trials (Correia et al., 2011). From the main effects it was also observed that the groups fed 44% SBM had a significant increase in jejunal crypt depth compared to the groups fed 48% SBM. This can be associated to the increase in dietary fiber in diets containing 44% SBM that causes the

increase in epithelial cell turnover due to the abrasive effect of dietary fiber, even when the enzyme has already modified the initial polymer structure of dietary fiber, provoking the need for more epithelial cell proliferation and crypt development (Montagne et al., 2003; Sittiya et al., 2020). The group fed 44% SBM without enzyme also increased the ileal villus height and the highest inclusion of β-mannanase decreased ileal villus to crypt ratio in the group containing 48% SBM. Such changes in ileal morphology are more pronounced in enzyme-free diets indicating the higher physical stimulation provoked by the presence of intact fiber particles in such groups. There are abrupt differences in the intestinal morphology in days 14 and 35. On day 14, duodenal villus was higher for the group fed 44% SBM without enzyme inclusion; however, on day 35, such group had the shortest duodenal villus height. Similarly, jejunal villus/crypt ratio was higher for the group fed 48% SBM with 0.5 ×; however, on day 35 this and all the other groups had the smallest ratio compared to the control group. Nevertheless, despite of the differences in the morphology of the different sections of the small intestine between day 14 and day 35, the results show a clear reduction in duodenal, jejunal, and ileal villus with higher inclusions of exogenous β-mannanase. Even though other researchers have reported similar results (Mehri et al., 2010; Park et al., 2019), there is a lack of explanation of the reason behind such changes. Therefore, is important to conduct future research to evaluate the dynamics of endogenous enzymes and their relationships with exogenous enzymes and their overall impact on intestinal development.

Gene expression and volatile fatty acids.

Different genes associated to immunity were evaluated on day 14 and 35 to assess the potential of soybean type and exogenous β-mannanase to modulate immune responses. None of the evaluated genes from the jejunal mucosa differed among dietary treatments at any time. Dietary treatments did not affect the expression of jejunal tight junction genes. However, on day 14 splenic

expression of IFGM was upregulated in treatments fed 44% SBM with 1 × enzyme. However, the expression of IFGM in splenic tissues disappeared on day 35. Interferon gamma plays an important function in the activation of antiviral proteins, α and β defensins, and is generally activated in the presence of virus and bacteria (Yuk et al., 2016). Therefore, the upregulation during the second week of age in groups fed 44% SBM might indicate changes in intestinal microbiota caused by the soluble portion of dietary fiber (Jha et al., 2019). Expression of cecal tonsil IL-1b and IL-10 was upregulated in the groups fed 48% SBM with 0.5 and 2 × enzyme inclusion. The expression of cecal tonsil IL-10 was higher for the groups fed 48% SBM with 0.5× enzyme inclusion but such upregulation was not observed on day 35. A higher expression of cecal tonsil IL-1b was observed on day 35 in the group fed 44% SBM without enzyme inclusion. The cecal tonsils are part of the gut-associated lymphoid tissue playing an important role in the modulation of immune responses in broilers (Li et al., 2014). The presence of IL-1b is generally associated to an increase in host immune and proinflammatory responses that leads to improvements in the production of immune-related molecules such as cytokines, adrenocorticotropin, and chemokines (Chen et al., 2016). Results from the main effects indicate that the upregulation of splenic IL-10 is associated to the interaction between 44% SBM and enzyme addition, where the presence of 44% SBM in the diet upregulates such gene. IL-10 is an anti-inflammatory cytokine that regulates the extend of inflammatory responses during bacterial, viral or any other infection (Couper et al., 2008; Moore et al., 2001). Similarly, the expression of cecal tonsil IL-1b is upregulated in the groups containing 44% SBM with lower enzyme inclusion. Since the expression of IL-1b is generally stimulated by microbes or microbial products (Wigley and Kaiser, 1996), the upregulation in diets without enzyme might indicate changes in intestinal microbiota due to higher dietary fiber in such diets. Similar to these results, Wils-Plotz et al., (2013) reported that broilers fed pectin had upregulated

expression of IL-1b compared to the control group. Interestingly, the same treatment with the higher upregulation of the IL-1b had the lowest expression of IL1-b on day 14. Furthermore, the upregulation of IL-1b and IL-10 in the group fed 48% SBM with 0.5 and 2 × enzyme addition might indicate a physiological response to reduce the inflammation in treatments with high expression of IL-1b due to changes in intestinal microbiota. The upregulation of IL-1b in the 44% SBM-fed groups might be also associated to the high pectin content in soyhulls that can be up to 30% as reported by Stein et al., 2008, which stimulates bacterial growth and subsequent gene regulation. The results from the present experiment, indicate the potential for 44% SBM to modulate the expression of immune proteins. In the case of IFGM and IL-1b, these cytokines play the role to mediate inflammation responses during disease or injury (Wigley and Kaiser). The upregulation of these genes in the 44% SBM might be associated to the effect of additional dietary fiber from soyhulls on the intestinal immunity and the break-down of galacto-mannans into mannan oligosaccharides by the action of the exogenous β-mannanase. In the case of the upregulation of IL-10 at the same time with in the cecal tonsils on d14, indicates the downregulation of immune responses since the expression of IL-10 is thought to begin after the proinflammatory immune response as a means to balance the production of cytokines such as IFGM, IL-1b, IL-2, and IL-6 (Scheller et al., 2011; Arendt et al., 2016). Other researchers have also reported the immune response of broilers fed diets supplemented with dietary fiber (Sadeghi et al., 2015). In some cases, additions of dietary fiber are regarded to be associated with the production of volatile fatty acids by intestinal microbiota which leads to changes in the expression of immune genes and immune status (Svihus et al., 2013). However, data from the present experiment indicate that additions of 3-4% soyhulls with or without β-mannanase in the diet does not promotes the formation of volatile fatty acids in the ileum (data not shown) nor in the ceca

(Table 6.10). The addition of exogenous β-mannanase has been regarded to result in the release of mannan oligosaccharides in commonly used corn and soybean meal diets (Huang et al., 2003) which is the possible way by which exerts immune responses. Mannan oligosaccharides have also been reported to positively influence other immune responses by increasing IgM and T-lymphocytes (Zou et al., 2006). And the data presented herein indicates the potential of exogenous β-mannanase to modulate immunity when added to normal corn and soybean meal diets.

Nutrient digestibility

All diets were formulated to be isocaloric and isonitrogenous and were mixed using 2 common basal diets (one using 44% and other using 48% SBM) to which different levels of β-mannanase were added. The digestibility of dry matter and energy was improved by the addition of 0.5 and 1× enzyme in the groups fed 48% SBM. Similar to these results, Cho and Kim, (2013) reported the improvement in energy and dry matter digestibility in in broilers fed diets with different energy levels and added exogenous β-mannanase. This is due to the ability of the enzyme to break down non-starch polysaccharides, namely mannans, increasing the release of glucose molecules and other molecules such as D-mannose as a source of energy (Saeed et al., 2019). Smaller fiber molecules can also be broken down into simple sugars and be absorbed in the small intestine or fermented (Ferreira et al., 2016). The presence of exogenous β-mannanase is considered to be beneficial due to the ability of the enzyme to cleave 4-β-D-mannan chains composed of galacto-glucomannans and galactomannans (McCleary, 1986). The digestibility of crude fat was reduced in the groups containing 44% SBM with or without enzyme addition. This can be explained by the ability of dietary fiber to bind fat molecules as reported by other researchers (Mirzaie et al., 2011). Therefore, the increase in dietary fiber in the groups containing 44% SBM results in fat binding and reduction in fat digestibility. However, such reduction in fat

digestibility was not necessarily associated with reduction in the digestibility of AME because of the small amounts of fat used in the experimental diets and due to the small differences in fat digestibility. The fact that the presence of β-mannanase in 44% SBM did not improve the crude fat digestibility is hard to explain and can be associated to the fact that oligosaccharides released during enzymatic cleavage can still bind fat molecules (Saeed et al., 2019). In general, the nutrient digestibility was improved by small additions of the enzyme (0.5 and 1×). This is because the enzyme cleaves the linkages of polysaccharides from the cell wall, releasing other nutrients that had been physically trapped (Hetland et al., 2003). However, high enzyme inclusions reduced growth performance and nutrient digestibility specially in the groups fed 44% SBM. Reductions in growth performance have already been reported when using high amounts of β-mannanase; however, the reason behind this phenomenon remains obscure (Mehri et al., 2010). This was also observed for the digestibility of crude protein and amino acids where the highest enzyme inclusion resulted in lower amino acid digestibility specially in the 44% SBM-fed groups. The fact that higher enzyme inclusions result in reductions in amino acid digestibility might be caused directly by the enzyme or indirectly by the release of other oligosaccharides with the ability to modulate microbiota and change digestibility patterns (Awad et al., 2015). The results from the main effects indicate that neither SBM type nor enzyme inclusion had a significant effect in the digestibility of essential amino acids; however, their interaction indicates that inclusions of the enzyme are more effective in 48% SBM containing groups than in those fed 44% SBM. The same was observed for the digestibility of nonessential amino acids. In fact, the digestibility of amino acids was higher in groups fed 44% SBM without enzyme but the addition of the enzyme helped improved the digestibility of amino acids in the 48% SBM-fed groups. Interestingly, the groups fed 48% SBM with 2 × enzyme inclusion had a significant reduction in weight gain, a numerical reduction in

feed intake (-3%) and an improved digestibility of essential amino acids. The reduction in feed intake can be associated to the reduction in body weight and also to the increase in the digestibility of essential amino acids to compensate for such reductions in intake. Similar to the results from the present experiment, Andreia et al., (2019) reported that broilers fed pelleted diets with restrictions at 90 and 85% resulted in increased digestibility of crude protein and digestible energy. Reports in other poultry species have also shown an increase in amino acid digestibility when including exogenous β-mannanase; however, such improvements in digestibility don't always lead to improvements in weight gain (Park et al., 2019). Therefore, care must be taken when using digestibility or other molecular parameters to describe the overall performance, since performance is the result of different metabolic processes and such processes are rapidly changed in poultry species as a means to offset physiological changes while keeping a balance.

Conclusions

Small additions of soybean hulls have positive implications in the growth performance, gene expression, intestinal morphology and nutrient digestibility in broiler diets. In the present experiment, hulled SBM (44% SBM) showed to numerically improve weight gain by about 3% compared to the control group. The same group with β-mannanase had higher expression of IL-10 in cecal tonsil tissues, similar jejunal villus height compared to the control group and improved ileal villus Therefore 44% SBM can be used in poultry diets with the adequate amendments in the nutrient matrix. Based on the results from the present study, it is concluded that inclusion of soyhulls or the utilization of hulled soybean meal can positively modulate the development of the intestinal tract and the expression of immune genes, resulting in improvements on growth performance when used in adequate amounts, namely 3-4% in the diet.

References

Adibmoradi, M., B. Navidshad, and M. F. Jahromi. 2016. The effect of moderate levels of finely ground insoluble fibre on small intestine morphology, nutrient digestibility and performance of broiler chickens. Ital. J. Anim. Sci. 15:310–317.

Amerah, A. M., V. Ravindran, R. G. Lentle, and D. G. Thomas. 2008. Influence of feed particle size on the performance, energy utilization, digestive tract development, and digesta parameters of broiler starters fed wheat- and corn-based diets. Poult. Sci. 87:2320–2328.

American Soybean Society. 2017. Soystats: A reference huide to important soybean facts and figures.

American Soybean Society. 2019. A Reference Guide To Soybean Facts and Figures, Soy Stats 2018. American Soybean Association, 1–36. Retrieved from https://ndsoygrowers.com/wp-content/uploads/2018/09/2018ASA-SoyStats.pdf

Andréia, M., J. C. Panisson, F. O. Marx, D. Surek, E. L. Krabbe, and A. Maiorka. 2019. Effect of pelleting and different feeding programs on growth performance, carcass yield, and nutrient digestibility in broiler chickens. Poult. Sci. 98:5497-5503.

Arendt. M. K., J. M. Sand, T. M. Marcone, and M. E. Cook. 2016. Interleukin-10 neutralizing antibody for detection of intestinal luminal levels and as a dietary additive in Eimeria challenged broiler chicks. Poult. Sci. 95:430-438

Awad,W.A., A. Smorodchenko, C. Hess, J. R. Aschenbach, A. Molnár, K. Dublecz, B. Khayal, E. E. Pohl, M. Hess. 2015. Increased intracellular calcium level and impaired nutrient absorption are important pathogenicity traits in the chicken intestinal epithelium during Campylobacter jejuni colonization. Appl. Microbiol. Biotechnol. 99:6431–6441.

Cao, B. H., X. P. Zhang, Y. M. Guo, Y. Karasawa, and T. Kumao. 2003. Effects of dietary

cellulose levels on growth, nitrogen utilization, retention time of diets in digestive tract and caecal microflora of chickens. Asian-Austr. J. Anim. Sci. 16:863–866.

Chen, W. T., W. Y. Huang, T. Chen, E. O. Salawu, D. Wang, Y. Z. Lee, C. S. Shih, X. Wang, and H. Sheng. 2016. Structure and function of chicken interleukin-1 beta mutants: Uncoupling of receptor binding and in vivo biological activity. Scie. Rep. 6:1–11.

Cho, J. H., and I. H. Kim. 2013. Effects of beta-mannanase supplementation in combination with low and high energy dense diets for growing and finishing broilers. Liv.Sci. 154:137–143.

Cobb-vantress. (2018). Broiler Management Guide. Accessed Jan. 2021. https://www.cobb-vantress.com/products/cobb500.

Cobb-Vantress. 2018. Broiler Performance and Nutrition Supplement Cobb500. Accessed Jan. 2021. http://www.cobb-vantress.com/docs/default-source/cobb-500-guides/cobb500-broiler-performance-nutrition-supplement-(english).pdf

Correia, M. A., K. Mazumder, J. A. Bras, S. J. Firbank, Y. Zhu, R. J. Lewis, W. S. York, C. M. Fontes, and H. J. Gilbert. 2011. Structure and function of an arabinoxylan-specific xylanase. J. Biol. Chem. 286:22510-22520.

Couper K. N., Blount D. G., Riley E. M. 2008. IL-10: the master regulator of immunity to infection. J. Immunol. 180:5771–5777

Dansky, L. M., and F. W. Hill. 1952. Application of chromic oxide indicator method to balance studies with growing chickens: two figures. J. Nutr. 47:449-459.

Daskiran, M., R. G. Teeter, D. Fodge, and H. Y. Hsiao. 2004. An evaluation of endo-β-D-mannanase (Hemicell) effects on broiler performance and energy use in diets varying in β-mannan content. Poult. Sci. 83:662–668.

Duke, G. E. 1982. Gastrointestinal motility and its regulation. Poult. Sci. 61:1245–1256.

Ferreira, H. C., M. I. Hannas, L. F. T. Albino, H. S. Rostagno, R. Neme, B. D. Faria, M. L. Xavier, and L. N. Rennó. 2016. Effect of the addition of β-mannanase on the performance, metabolizable energy, amino acid digestibility coefficients, and immune functions of broilers fed different nutritional levels. Poult. Sci. 95:1848–1857.

Friesen, O. D., W. Guenter, R. R. Marquardt, and B. A. Rotter. 1992. The effect of enzyme supplementation on the apparent metabolizable energy and nutrient digestibilities of wheat, barley, oats, and rye for the young broiler chick. Poult. Sci. 71:1710–1721.

Gonzalez-Alvarado, J. M., E. Jimenez-Moreno, R. Lazaro, G. Mateos. 2007. Effect of Type of Cereal , Heat Processing of the Cereal , and Inclusion of Fiber in the Diet on Productive Performance and Digestive Traits of Broilers. Poult. Sci. 86:1705-1715.

González-Alvarado, J. M., E. Jiménez-Moreno, D. G. Valencia, R. Lázaro, R., and G. Mateos. 2008. Effects of fiber source and heat processing of the cereal on the development and pH of the gastrointestinal tract of broilers fed diets based on corn or rice. Poult. Sci. 87:1779–1795.

Hetland, H., B. Svihus, and A. Krogdahl. 2003. Effects of oat hulls and wood shavings on digestion in broilers and layers fed diets based on whole or ground wheat. Br. Poult. Sci. 44:275–282.

Hirano T. Interleukin 6. In: AE Thomson (editor). The Cytokine Handbook 3rd edition. Academic Press, San Diego. 1998; p.197-227.

Hsiao, H. Y., D. M., Anderson, and N. M. Dale. 2006. Levels of β-mannan in soybean meal. Poult. Sci. 8:1430-1432

Huang, X. W., X. S. Liu, and F. Q. Xu. 2003. The effect of β- mannanase on growth performance of growing pigs. Feed Res. 2003:29–31.

Jha, R., J. M. Fouhse, U. P. Tiwari, L. Li, B. P. Willing. 2019. Dietary fiber and intestinal health of monogastric animals. Fr. Vet. Sci. 6:1–12.

Jiménez-Moreno, E., J. M. González-Alvarado, A. González-Serrano, R. Lázaro, G. Mateos. 2009. Effect of dietary fiber and fat on performance and digestive traits of broilers from one to twenty-one days of age. Poult. Sci. 88:2562–2574.

Johnson, L., and K. Smith. 2004. Fact sheet: Soybean processing. The Soybean Meal Information Center. 3:489–496.

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

Karimi, K., and M. Zhandi. 2014. The effect of beta-mannanase and beta-glucanase on small intestine morphology in male broilers fed diets containing various levels of metabolizable energy. J. Appl. Anim. Res. 43:324–329.

Lourenco, J. M., T. J. Kierran, D. S. Seidel, T. C. Glenn, M. D. da Silveira, T. R. Callaway, R. L. Stewarr Jr. 2020. Comparison of the ruminal and fecal microbiotas in beef calves cupplemeneted or not with concentrate. PLoS ONE. 15(4)

Lee, J. T., S. Connor-Appleton, C. A. Bailey, and A. L. Cartwright. 2005. Effects of guar meal by-product with and without β-mannanase Hemicell on broiler performance. Poult. Sci. 84: 1261–1267.

Li, J., Z. Wang, J. Cao, Y. L. Dong, and Y. X. Chen. 2014. Role of monochromatic light on development of cecal tonsil in young broilers. Anatom. Record. 297:1331–1337.

Mccleary, B. V. 1986. Enzymatic modification of plant polysaccharides. Int. J. Biol. Macromol. 8:349-354.

Mehri, M., M. Adibmoradi, A. Samie, and M. Shivazad. 2010. Effects of β-mannanase on broiler performance, gut morphology and immune system. A. J. Biotechnol. 9:6221-6228.

Mirzaie, S., M. Zaghari, S. Aminzadeh, and M. Shivazad. 2011. The effects of non-starch polysaccharides content of wheat and xylanase supplementation on the intestinal amylase, aminopeptidase and lipase activities, ileal viscosity and fat digestibility in layer diet. I. J. Biotechnol. 10:208-214.

Montagne, L., J. R. Pluske, and D. J. Hampson. 2003. A review of interactions between dietary fibre and the intestinal mucosa, and their consequences on digestive health in young non-ruminant animals. Anim. F. Sci. Technol. 108:95–117.

Moore K. W., de Waal Malefyt R., Coffman R. L., O'Garra A. 2001. Interleukin-10 and the interleukin-10 receptor. Annu. Rev. Immunol. 19:683–765.

Park, J., S. Jung, and J. B. Carey. 2019. Effects of a commercial beta-mannanase product on growth performance, intestinal histomorphology, bone and body composition, and amino acid digestibility in white pekin ducks. J. Appl. Poult. Res. 28:63-71.

Rezaei, M., M. A. Karimi Torshizi, H. Wall, and E. Ivarsson. 2018. Body growth, intestinal morphology and microflora of quail on diets supplemented with micronised wheat fibre. Br. Poult. Sci. 59: 422–429.

Sadeghi, A., M. Toghyani, and A. Gheisari. 2015. Effect of various fiber types and choice feeding of fiber on performance, gut development, humoral immunity, and fiber preference in broiler chicks. Poult. Sci. 94:2734–2743.

Saeed, M., T. Ayaşan, M. Alagawany, M. E. A. El-Hack, M. A. Abdel-Latif, and A. K. Patra. 2019. The role of β-Mannanase (Hemicell) in improving poultry productivity, health and environment. Rev. Bras. Cien. Avi. 21:1-8

Scheller J., A. Chalaris, D. Schmidt-Arras, and S. Rose-John. 2011. The pro- and anti-inflammatory properties of the cytokine interleukin-6. Biochim. Biophys. Acta, 1813:878-888.

Sittiya, J., K. Yamauchi, W. Nimanong, and N. Thongwittaya. 2020. Influence of levels of dietary fiber sources on the performance, carcass traits, gastrointestinal tract development, fecal ammonia nitrogen, and intestinal morphology of broilers. Rev. Bras. Cienc. Avi. 22:1–8.

Sklan, D., A. Smirnov, I. Plavnik. 2003. The effect of dietary fibre on the small intestines and apparent digestion in the turkey. Br. Poult. Sci. 44:735–740.

Stein, H. H., L. L. Berger, J. K. Drackley, G.C. Fahey, Jr, D. C. Hernot, and C. M. Parsons. 2008. Nutritional properties and feeding values of soybeans and their co-products. Pages 613–660 in Soybeans, Chemistry, Production, Processing, and Utilization. L. A. Johnson, P. J. White and R. Galloway, eds. AOCS Press, Urbana, IL.

Svihus, B., M. Choct, and H. L. Classen. 2013. Function and nutritionalroles of the avian caeca: a review. W. Poult. Sci. J. 69:249-264.

Tejeda, O. J., and W. K. Kim. 2020. The effects of cellulose and soybean hulls as sources of dietary fiber on the growth performance, organ growth, gut histomorphology, and nutrient digestibility of broiler chickens. Poult. Sci. 99:6828–6836.

Viveros, A., A. Brenes, M. Pizarro, and M. Castano. 1994. Effect of enzyme supplementation of a diet based on barley, and autoclave treatment, on apparent digestibility, growth performance and gut morphology of broilers. Anim. F. Sci. Technol. 48:237–251.

Waldrup, P. W. 2006. Soybean meal demand. Fact sheet. Accessed May 2021. https://www.soymeal.org/soy-meal-articles/soybean-meal-demand/

Walugembe M, J. C. Hsieh, N. J. Koszewski, S. J. Lamont, M. E. Persia, M. Rothschild. 2015. Effects of dietary fiber on cecal short-chain fatty acid and cecal microbiota of broiler and laying-hen chicks. Poult. Sci. 94:2351-9.

Wigley, P, and P. Kaiser. 2003. Avian cytokines in health and disease. Braz. J. Poult. Sci. 5:1-14

Wils-Plotz, E. L., M. C. Jenkins, and R. N. Dilger. 2012. Modulation of the intestinal environment, innate immune response, and barrier function by dietary threonine and purified fiber during a coccidiosis challenge in broiler chickens. Poult. Sci. 92:735-745.

Yuk S. S., Lee D. H., Park J. K., Tseren-Ochir E. O., Kwon J. H., Noh J. Y., Lee J. B., Park S. Y., Choi I. S., and Song C. S. 216. Pre-immune state induced by chicken interferon gamma inhibits the replication of H1N1 human and H9N2 avian influenza viruses in chicken embryo fibroblasts. Virol. J. 13:71.

Zou, X. T., X. J. Qiao, Z. R. Xu. 2006. Effect of β-mannanase (Hemicell) on growth performance and immunity of broilers. Poult. Sci. 85:2176–2179.

Table 6.1. Proximate analyses of the nutrient composition of soybean hulls

Item	Value
GE (Kcal/Kg)	3,698
AMEn (Kcal/kg)	658
Dry Matter (%)	87.95
Crude Protein (%)	16.3
Crude Fiber (%)	35.8
Calcium (%)	0.88
Phosphorus (%)	0.55
nPP (%)	0.37

Table 6.2. Amino acid content, digestibility (%) and digestible amino-acid content of Soybean Hulls based on cecectomized rooster assay.

Amino Acid	Percent Amino acid	Digestibility (%)	Digestible Amino acid content (%)
Alanine	0.450	39.386	0.177
Arginine	0.480	68.344	0.328
Aspartic acid	0.950	54.040	0.513
Cysteine	0.160	41.461	0.066
Glutamic acid	1.140	51.294	0.585
Histidine	0.260	46.506	0.121
Isoleucine	0.400	43.850	0.175
Leucine	0.660	52.328	0.345
Lysine	0.730	52.738	0.383
Methionine	0.130	57.583	0.075
Phenylalanine	0.390	53.601	0.209
Proline	0.550	55.592	0.306
Serine	0.530	49.335	0.261
Threonine	0.360	50.722	0.183
Tryptophan	0.060	56.100	0.034
Tyrosine	0.360	46.732	0.168
Valine	0.460	33.951	0.156

Table 6.3. Ingredient and calculated nutrient composition of diets fed to male Cobb broilers from 1 to 42 d of age[1]

	Starter (d1-14)		Grower (d15-28)		Finisher (d29-42)	
Ingredient	**SBM 48%**	**SBM 44%**	**SBM 48%**	**SBM 44%**	**SBM 48%**	**SBM 44%**
Corn	62.53	57.34	65.61	60.41	68.63	64.60
SMB 48%	33.00	32.38	29.66	29.19	25.95	25.47
Soyhulls[2]	-	4.00	-	3.60		3.11
Soybean oil	0.33	2.17	1.01	2.85	1.71	3.15
Defluorinated phosphate	1.64	1.68	1.39	1.42	1.31	1.33
limestone	0.64	0.53	0.63	0.52	0.62	0.53
DL-met	0.31	0.33	0.25	0.27	0.27	0.28
L-Thr	0.10	0.12	0.02	0.03	0.04	0.05
Lysine HCl	0.24	0.26	0.20	0.21	0.22	0.23
Sodium chloride	0.15	0.15	0.25	0.25	0.20	0.20
Trace minerals[3]	0.15	0.15	0.15	0.15	0.15	0.15
Vitamins[4]	0.05	0.05	0.05	0.05	0.05	0.05
Cr2O3	0.30	0.30	0.00	0.00	0.30	0.30
Sand	0.55	0.55	0.79	1.05	0.55	0.55
Total	100	100	100	100	100	100
Calculated Nutrient Composition[5]						
AME, Kcal/kg	2,975	2,975	3,050	3,050	3,125	3,125
Protein	21.00	21.00	19.50	19.50	18.00	18.00
Dry matter	88.3	88.5	88.6	88.7	88.7	88.8
Crude fiber	2.18	3.49	2.14	3.31	2.08	3.11
Calcium	0.90	0.90	0.80	0.80	0.76	0.76
Available phosphorus	0.45	0.45	0.40	0.40	0.38	0.38
Av. Met	0.63	0.63	0.55	0.56	0.55	0.55
Av. TSAA	0.91	0.91	0.82	0.82	0.80	0.80
Av. Lys	1.22	1.22	1.10	1.10	1.02	1.02
Av. Trp	0.24	0.23	0.22	0.22	0.20	0.19
Av. Thr	0.83	0.83	0.70	0.70	0.66	0.66

[1]Starter was provided as crumbles, grower and finisher were provided as pellets
[2]Soyhulls was added to regular 48% CP SBM at 12% to decrease the CP level to 44%
[3]Mineral premix includes per kg of premix: Ca, 0.72 g; Mn, 3.04 g; Zn, 2.43 g; Mg, 0.61 g; Fe, 0.59 g; Cu, 22.68 g; I, 22.68 g; Se, 9.07 g.
[4]Vitamin premix provided the following per kilogram of DSM premix: Vit. A, 2,204,586 IU; Vit. D_3, 200,000 ICU; Vit. E, 2,000 IU; Vit. B12, 2 mg; Biotin, 20 mg; Menadione, 200 mg; Thiamine, 400 mg; Riboflavin, 800 mg; d-Pantothenic Acid, 2,000 mg; Vit. B6, 400 mg; Niacin, 8,000 mg; Folic Acid, 100 mg; Choline, 34,720 mg
[5]All values are reported as percentages unless noted otherwise

Table 6.4. Primer pairs used for RT-qPCR analyses

Gene[1]	Gene bank identification	Primer sequence, forward/reverse	Product size (bp)
GAPDH[2]	NC_052532.1	GCTAAGGCTGTGGGGAAAGT/ TCAGCAGCAGCCTTCACTAC	161
CLDN1	NM_001013611.2	TGGAGGATGACCAGGTGAAGA/ CGAGCCACTCTGTTGCCATA	115
OCLN	XM_026041453.1	ACGGCAGCACCTACCTCAA/ GGCGAAGAAGCAGATGAG	122
IL-1b	HQ329098.1	TGCCTGCAGAAGAAGCCTCG/ GACGGGCTCAAAAACCTCCT	204
IL-6	NM_204628	AAAGCAGAACGTCGAGTC/ CTTCAGATTGGCGAGGAG	133
Pept1	KF366603.1	CCCCTGAGGAGGATCACTGTT/ CAAAAGAGCAGCAGCAACGA	66

[1]GAPDH, glyceraldehyde-3-phosphate dehydrogenase; CLDN1, claudin 1; OCLN, Occludin; IL-1b, interleukin-1b; IL-6, interleukin 6; Pept-1, peptide transporter 1.
[2]Housekeeping gene

Table 6.5. Effects of SBM type and β-mannanase addition on the growth performance of male broilers reared to 42 days of age[1]

		BW gain (g/bird)			Feed intake (g/bird)			Feed : BW gain		
SBM type[2]	**Enzyme level**	**Day 14**	**Day 35**	**Day 42**	**Day 14**	**Day 35**	**Day 42**	**Day 14**	**Day 35**	**Day 42**
48% CP	**0 IU**	0.452^{a}	1.88^{a}	3.66^{ab}	0.576	2.64	5.48	1.28^{ab}	1.45^{ab}	1.59
48% CP	**21,750 IU**	0.448^{ab}	1.86^{ab}	3.80^{a}	0.577	2.63	5.50	1.29^{ab}	1.46^{abc}	1.56
48% CP	**43,500 IU**	0.427^{abc}	1.85^{ab}	3.70^{ab}	0.561	2.60	5.50	1.32^{ab}	1.45^{ab}	1.58
48% CP	**87,000 IU**	0.409^{c}	1.77^{b}	3.51^{b}	0.551	2.55	5.34	1.35^{b}	1.49^{b}	1.61
44% CP	**0 IU**	0.451^{a}	1.90^{a}	3.76^{a}	0.568	2.64	5.62	1.26^{a}	1.44^{a}	1.59
44% CP	**21,750 IU**	0.451^{a}	1.87^{ab}	3.60^{ab}	0.573	2.65	5.46	1.27^{ab}	1.45^{ab}	1.59
44% CP	**43,500 IU**	0.453^{a}	1.80^{ab}	3.63^{ab}	0.578	2.65	5.49	1.28^{ab}	1.46^{abc}	1.60
44% CP	**87,000 IU**	0.419^{bc}	1.81^{ab}	3.63^{ab}	0.557	2.59	5.48	1.33^{ab}	1.48^{bc}	1.59
Standard Error		0.007	0.02	0.05	0.009	0.03	0.05	0.02	0.01	0.02
P-value		<0.001	0.008	0.002	0.322	0.240	0.052	0.008	<0.001	0.464
Main effects										
SBM type	**48% CP**	0.434	1.84	3.70	0.566	2.60	5.45	1.31	1.46	1.58
	44% CP	0.444	1.86	3.67	0.569	2.63	5.51	1.28	1.46	1.59
Enzyme level	**0 IU**	0.451^{a}	1.89^{a}	3.71^{a}	0.572	2.60	5.55	1.28^{a}	1.44^{a}	1.59
	21,750 IU	0.450^{a}	1.87^{a}	3.70^{a}	0.575	2.64	5.48	1.28^{a}	1.45^{a}	1.57
	43,500 IU	0.440^{a}	1.86^{a}	3.67^{ab}	0.570	2.62	5.49	1.30^{ab}	1.46^{a}	1.59
	87,000 IU	0.414^{b}	1.79^{b}	3.57^{b}	0.554	2.57	5.41	1.34^{b}	1.49^{b}	1.60
Source of variation (P-value)										
SBM type (T)		0.060	0.215	0.715	0.645	0.209	0.124	0.085	0.460	0.389
Enzyme level (E)		<0.001	<0.001	0.013	0.126	0.081	0.067	0.001	<0.001	0.304
T × E		0.228	0.937	0.003	0.541	0.842	0.175	0.900	0.405	0.518

[1]Values are the least-square means of 8 replicate pens per treatment, each pen with 20 birds.
[2]Soybean meal 44% was formulated by adding soyhulls at a ratio of 12%, refer to Table 6.3.
[a-d]Means within a column not sharing a common superscript differ significantly (P < 0.05).

Table 6.6. Effects of SBM type and β-mannanase addition on the intestinal morphology (μm) of male broilers at 14 d of age[1]

		Duodenum			Jejunum			Ileum		
SBM type[2]	**Enzyme level**	**Villus (V)**	**Crypt (C)**	**V:C ratio**	**Villus (V)**	**Crypt (C)**	**V:C ratio**	**Villus (V)**	**Crypt (C)**	**V:C ratio**
48% CP	**0 IU**	1907^{abc}	177^{ab}	11.28	1075^{abc}	130^{b}	8.68^{ab}	702^{b}	122^{ab}	6.26^{a}
48% CP	**21,750 IU**	1833^{bcd}	163^{ab}	11.86	1199^{a}	130^{b}	9.39^{a}	694^{bc}	128^{ab}	5.75^{ab}
48% CP	**43,500 IU**	1902^{abc}	165^{ab}	11.90	1033^{bc}	134^{b}	8.22^{ab}	798^{a}	138^{a}	6.09^{a}
48% CP	**87,000 IU**	1804^{cd}	167^{ab}	11.16	1049^{bc}	140^{ab}	7.77^{b}	626^{cd}	119^{b}	5.43^{ab}
44% CP	**0 IU**	1976^{a}	177^{ab}	11.61	1118^{abc}	136^{b}	8.67^{ab}	674^{bcd}	129^{ab}	5.53^{ab}
44% CP	**21,750 IU**	1947^{ab}	164^{ab}	12.51	1129^{ab}	153^{a}	7.72^{b}	743^{ab}	123^{ab}	6.28^{a}
44% CP	**43,500 IU**	1847^{abcd}	184^{a}	10.77	992^{c}	133^{b}	7.71^{b}	621^{d}	129^{ab}	5.02^{b}
44% CP	**87,000 IU**	1755^{d}	158^{b}	11.42	1071^{abc}	132^{b}	8.36^{ab}	627^{cd}	123^{ab}	5.36^{ab}
Standard Error		32	5	0.38	33	4	0.32	17	4	0.22
P-value		<0.01	<0.01	0.054	<0.01	<0.01	<0.01	<0.01	0.04	<0.01
Main effects										
SBM type	**48% CP**	1862	168	11.55	1089	134	8.51	705^{a}	127	5.88^{a}
	44% CP	1881	171	11.58	1078	138	8.11	667^{b}	126	5.55^{b}
Enzyme level	**0 IU**	1942^{a}	176^{a}	11.44	1097^{ab}	133	8.67	688^{a}	125^{ab}	5.89^{ab}
	21,750 IU	1890^{a}	164^{b}	12.18	1164^{a}	142	8.55	718^{a}	125^{ab}	6.01^{a}
	43,500 IU	1874^{a}	174^{ab}	11.34	1013^{c}	133	7.96	710^{a}	133^{a}	5.55^{ab}
	87,000 IU	1780^{b}	162^{b}	11.29	1060^{bc}	136	8.06	626^{b}	121^{b}	5.40^{b}
Source of variation (P-value)										
SBM type (T)		0.376	0.426	0.910	0.590	0.092	0.078	<0.01	0.815	0.032
Enzyme level (E)		<0.01	<0.01	0.063	<0.01	0.104	0.065	<0.01	0.021	0.016
T × E		0.013	0.037	0.086	0.209	<0.01	<0.01	<0.01	0.178	<0.01

[1]Values are the least-square means of 8 replicate pens per treatment, each pen with 20 birds.
[2]Soybean meal 44% was formulated by adding soyhulls at a ratio of 12%, refer to Table 6.3.
[a-d]Means within a column not sharing a common superscript differ significantly (P < 0.05).

Table 6.7. Effects of SBM type and β-mannanase addition on the intestinal morphology of male broilers at 35 days of age[1]

		Duodenum			Jejunum			Ileum		
SBM type[2]	**Enzyme level**	**Villus (V)**	**Crypt (C)**	**V:C ratio**	**Villus (V)**	**Crypt (C)**	**V:C ratio**	**Villus (V)**	**Crypt (C)**	**V:C ratio**
48% CP	**0 IU**	2705^{ab}	190	14.72^{ab}	1832^{a}	186	10.64^{a}	945^{ab}	163	5.98^{ab}
48% CP	**21,750 IU**	2643^{b}	198	13.98^{b}	1543^{b}	203	8.09^{b}	874^{b}	157	5.77^{ab}
48% CP	**43,500 IU**	2796^{ab}	199	14.88^{ab}	1548^{b}	210	7.94^{b}	880^{b}	157	5.84^{ab}
48% CP	**87,000 IU**	2691^{ab}	193	14.62^{ab}	1508^{b}	183	8.49^{b}	878^{b}	191	5.32^{b}
44% CP	**0 IU**	2625^{b}	196	14.07^{ab}	1848^{a}	211	9.32^{ab}	1043^{a}	204	5.90^{ab}
44% CP	**21,750 IU**	2719^{ab}	178	16.25^{a}	1522^{b}	196	8.29^{b}	974^{ab}	160	6.33^{a}
44% CP	**43,500 IU**	2856^{a}	188	15.70^{ab}	1599^{b}	214	7.65^{b}	899^{b}	168	5.62^{ab}
44% CP	**87,000 IU**	2761^{ab}	189	15.10^{ab}	1613^{b}	221	8.52^{b}	959^{ab}	155	6.38^{a}
Standard Error		50	6	0.54	51	11	0.42	27	14	0.23
P-value		0.010	0.221	0.036	<0.01	0.124	<0.01	<0.01	0.056	0.010
Main effects										
SBM type	**48% CP**	2708	195	14.60	1608	195^{b}	8.79	894^{b}	167	5.73^{b}
	44% CP	2740	188	15.30	1645	211^{a}	8.45	968^{a}	172	6.06^{a}
Enzyme level	**0 IU**	2665^{b}	193	14.40	1840^{a}	198	9.98^{a}	994^{a}	184	5.94
	21,750 IU	2681^{b}	188	15.11	1533^{b}	200	8.19^{b}	924^{b}	159	6.05
	43,500 IU	2826^{a}	193	15.30	1574^{b}	212	7.80^{b}	889^{b}	162	5.73
	87,000 IU	2726^{ab}	191	14.86	1560^{b}	202	5.51^{b}	918^{b}	173	5.85
Source of variation (P-value)										
SBM type (T)		0.3492	0.080	0.0514	0.286	0.043	0.225	<0.01	0.597	0.030
Enzyme level (E)		0.0029	0.799	0.3493	<0.01	0.572	<0.01	<0.01	0.224	0.479
T × E		0.2919	0.149	0.0483	0.633	0.137	0.213	0.340	0.036	0.010

[1]Values are the least-square means of 8 replicate pens per treatment, each pen with 20 birds.
[2]Soybean meal 44% was formulated by adding soyhulls at a ratio of 12%, refer to Table 6.3.
[a-d]Means within a column not sharing a common superscript differ significantly (P < 0.05).

Table 6.8. Effects of SBM type and β-mannanase addition on the gene expression of male broilers at 14 d of age[1]

		Jejunal scratch		Spleen			Cecal tonsil		
SBM type[2]	**Enzyme level**	**Occludin**	**Claudin**	**IFGM**	**IL-1b**	**IL-10**	**IFGM**	**IL-1b**	**IL-10**
48% CP	**0 IU**	1.00	1.00	1.00^{c}	1.00	1.00	1.00	1.00^{b}	1.00^{c}
48% CP	**21,750 IU**	1.32	1.57	1.18^{bc}	0.65	0.44	3.34	2.93^{a}	2.06^{a}
48% CP	**43,500 IU**	0.61	0.73	2.32^{abc}	0.62	1.27	1.04	0.97^{b}	1.02^{bc}
48% CP	**87,000 IU**	1.21	0.78	1.05^{c}	0.52	1.01	2.53	2.84^{a}	1.88^{ab}
44% CP	**0 IU**	1.70	1.13	2.75^{ab}	0.48	1.10	1.03	0.91^{b}	0.73^{c}
44% CP	**21,750 IU**	1.04	0.94	1.21^{bc}	1.04	0.43	1.68	1.60^{ab}	1.07^{bc}
44% CP	**43,500 IU**	1.46	1.34	3.35^{a}	0.92	1.23	2.13	1.62^{ab}	1.05^{bc}
44% CP	**87,000 IU**	0.67	0.71	1.53^{bc}	0.55	0.69	2.81	1.87^{ab}	1.53^{abc}
Standard Error		0.33	0.31	0.43	0.20	0.27	0.69	0.59	0.34
P-value		0.277	0.483	<0.01	0.236	0.140	0.070	0.038	0.034
Main effects									
SBM type	**48% CP**	1.03	1.02	1.39^{b}	0.70	0.93	1.98	1.94	1.49
	44% CP	1.22	1.03	2.21^{a}	0.75	0.86	1.91	1.50	1.10
Enzyme level	**0 IU**	1.35	1.07	1.88^{ab}	0.74	1.05	1.02^{b}	0.95^{b}	0.86^{b}
	21,750 IU	1.18	1.25	1.19^{b}	0.84	0.43	2.51^{ab}	2.27^{ab}	1.57^{ab}
	43,500 IU	1.03	1.04	2.84^{a}	0.77	1.25	1.59^{ab}	1.29^{ab}	1.04^{ab}
	87,000 IU	0.94	0.74	1.29^{b}	0.54	0.85	2.67^{a}	2.36^{a}	1.71^{a}
Source of variation (P-value)									
SBM type (T)		0.445	0.960	<0.01	0.728	0.718	0.883	0.246	0.074
Enzyme level (E)		0.637	0.452	<0.01	0.455	0.020	0.026	0.017	0.019
T × E		0.106	0.268	0.131	0.100	0.862	0.193	0.255	0.434

[1]Values are the least-square means of 8 replicate pens per treatment, each pen with 20 birds.
[2]Soybean meal 44% was formulated by adding soyhulls at a ratio of 12%, refer to Table 6.3.
[a-d]Means within a column not sharing a common superscript differ significantly (P < 0.05).

Table 6.9. Effects of SBM type and β-mannanase addition on the gene expression of male broilers at 35 d of age[1]

		Jejunal scratch			Spleen			Cecal tonsil		
SBM type[2]	Enzyme level	Occludin	Claudin	Pept-1	IFGM	IL-1b	IL-6	IFGM	IL-1b	IL-6
48% CP	**0 IU**	1.00	1.00	1.00	1.00	1.00	1.00^{d}	1.00	1.00^{b}	1.00
48% CP	**21,750 IU**	1.13	1.23	0.91	2.94	0.74	1.30^{cd}	2.48	1.84^{ab}	1.15
48% CP	**43,500 IU**	1.82	2.18	1.68	2.36	0.65	1.57^{bcd}	1.97	0.85^{b}	0.63
48% CP	**87,000 IU**	0.88	2.07	1.01	2.93	1.03	2.46^{abc}	1.13	1.07^{ab}	2.75
44% CP	**0 IU**	0.90	1.47	1.05	3.51	0.94	2.58^{ab}	2.35	2.25^{a}	1.47
44% CP	**21,750 IU**	1.00	0.69	1.16	1.68	1.00	1.63^{bcd}	2.01	1.00^{b}	1.76
44% CP	**43,500 IU**	0.65	0.82	0.53	4.37	0.95	2.93^{a}	1.40	1.03^{b}	1.69
44% CP	**87,000 IU**	1.27	3.35	2.00	1.84	0.77	1.13^{d}	1.99	0.67^{b}	1.08
Standard Error		0.28	0.87	0.46	1.04	0.20	0.44	0.62	0.28	0.61
P-value		0.269	0.318	0.986	0.219	0.777	0.016	0.470	0.002	0.332
Main effects										
SBM type	**48% CP**	1.21	1.62	1.15	2.31	0.85	1.58	1.64	1.19	1.38
	44% CP	0.95	1.58	1.19	2.85	0.92	2.07	1.94	1.24	1.50
Enzyme level	**0 IU**	0.95	1.24	1.03	2.25	0.97	1.79	1.67	1.63^{a}	1.23
	21,750 IU	1.07	0.96	1.04	2.31	0.87	1.46	2.25	1.42^{ab}	1.46
	43,500 IU	1.23	1.50	1.10	3.36	0.80	2.25	1.69	0.94^{b}	1.16
	87,000 IU	1.08	2.71	1.50	2.39	0.90	1.80	1.56	0.87^{b}	1.91
Source of variation (P-value)										
SBM type (T)		0.2153	0.9535	0.9131	0.416	0.641	0.124	0.468	0.807	0.782
Enzyme level (E)		0.8150	0.1561	0.6949	0.620	0.833	0.3711	0.617	0.031	0.573
T × E		0.0715	0.4080	0.1599	0.090	0.380	0.007	0.234	0.003	0.108

[1]Values are the least-square means of 8 replicate pens per treatment, each pen with 20 birds.
[2]Soybean meal 44% was formulated by adding soyhulls at a ratio of 12%, refer to Table 6.3.
[a-d]Means within a column not sharing a common superscript differ significantly ($P < 0.05$).

Table 6.10. Effects of SBM type and β-mannanase addition on the cecal production of volatile fatty acids (mM) at d35[1]

SBM type[2]	Enzyme level	Acetate	Propionate	Isobutyrate	Butyrate	Isovalerate	Valerate	Total VFA
48% CP	**0 IU**	72.6	9.8	0.64	16.0	1.04	1.60	101.7
48% CP	**21,750 IU**	66.2	9.5	0.59	15.9	1.06	1.50	94.8
48% CP	**43,500 IU**	65.0	10.5	0.70	13.9	1.12	1.50	92.7
48% CP	**87,000 IU**	71.0	10.1	0.54	14.5	0.88	1.67	98.8
44% CP	**0 IU**	67.0	9.5	0.53	17.1	0.90	1.42	96.5
44% CP	**21,750 IU**	64.5	10.1	0.65	16.5	0.92	1.53	94.2
44% CP	**43,500 IU**	71.6	11.1	0.53	18.0	0.93	1.64	103.7
44% CP	**87,000 IU**	63.8	9.4	0.54	14.5	0.96	1.47	90.8
Standard Error		6.4	0.6	0.08	2.2	0.12	0.10	8.8
P-value		0.951	0.542	0.742	0.886	0.839	0.655	0.968
Main effects								
SBM type	**48% CP**	68.7	10.0	0.62	15.1	1.03	1.56	97.0
	44% CP	66.7	10.0	0.59	16.5	0.93	1.51	96.3
Enzyme level		69.8	9.7				1.5	99.1
	0 IU			0.58	16.5	0.97		
	21,750 IU	65.4	9.8	0.62	16.2	0.99	1.5	94.5
	43,500 IU	68.3	10.8	0.61	15.9	1.03	1.6	98.2
	87,000 IU	67.4	9.8	0.59	14.5	0.92	1.6	94.8
Variation (P-value)								
SBM type (T)		0.668	0.931	0.556	0.351	0.264	0.543	0.913
Enzyme (E)		0.916	0.240	0.964	0.801	0.849	0.817	0.934
T × E		0.708	0.640	0.310	0.790	0.720	0.302	0.716

[1]Values are the least-square means of 8 replicate birds per treatment.
[a-d]Means within a column not sharing a common superscript differ significantly ($P < 0.05$).

Table 6.11. Effects of SBM type and β-mannanase addition on nutrient digestibility of male broilers at 42 d of age[1]

SBM type[2]	Enzyme level	DM[3], %	Energy, kcal/kg	ADF[3], %	Crude fat, %
48% CP	**0 IU**	74.17^{bc}	3496^{a}	24.23	93.33^{ab}
48% CP	**21,750 IU**	79.06^{a}	3541^{a}	37.23	94.72^{a}
48% CP	**43,500 IU**	79.01^{a}	3591^{a}	33.35	94.80^{a}
48% CP	**87,000 IU**	77.80^{ab}	3474^{a}	36.45	93.47^{ab}
44% CP	**0 IU**	76.72^{abc}	3478^{a}	30.79	91.99^{bc}
44% CP	**21,750 IU**	77.09^{abc}	3463^{ab}	28.06	92.75^{b}
44% CP	**43,500 IU**	77.60^{ab}	3526^{a}	38.55	92.63^{b}
44% CP	**87,000 IU**	73.10^{c}	3313^{b}	31.38	90.98^{c}
Standard Error		1.00	35.1	3.60	0.36
P-value		<0.01	<0.01	0.141	<0.01
Main effects					
SBM type	**48% CP**	77.51	3525^{a}	32.20	94.07^{a}
	44% CP	76.13	3445^{b}	32.82	92.09^{b}
Enzyme level	**0 IU**	75.44^{b}	3487^{ab}	27.51	92.66^{b}
	21,750 IU	78.08^{ab}	3502^{a}	32.65	93.73^{a}
	43,500 IU	78.31^{a}	3558^{a}	35.95	93.72^{a}
	87,000 IU	75.45^{b}	3394^{b}	33.92	92.22^{b}
Source of variation (P-value)					
SBM type (T)		0.059	<0.01	0.813	<0.01
Enzyme level (E)		<0.01	<0.01	0.158	<0.01
T × E		<0.01	0.254	0.100	0.455

[1]Values are the least-square means of 8 replicate pens per treatment, each pen with 20 birds.
[2]Soybean meal 44% was formulated by adding soyhulls at a ratio of 12%, refer to Table 6.3.
[3]DM = dry matter; ADF = acid detergent fiber.
[a-d]Means within a column not sharing a common superscript differ significantly ($P < 0.05$).

Table 6.12. Effects of SBM type and β-mannanase addition on essential AA digestibility of male broilers at 42 d of age[1]

SBM type[2]	Enzyme level	CP, %	Met, %	Lys, %	Thr, %	Trp, %	Val, %	Arg, %	Phe, %	Leu, %	Ile, %
48% CP	**0 IU**	82.72^{bc}	92.66^{bc}	88.45^{b}	78.03^{c}	87.02^{b}	81.58^{c}	90.37^{c}	84.49^{c}	83.83^{c}	82.87^{c}
48% CP	**21,750 IU**	84.76^{abc}	94.92^{abc}	91.68^{ab}	82.57^{ab}	88.57^{ab}	85.59^{ab}	93.35^{abc}	88.30^{ab}	87.64^{ab}	86.68^{ab}
48% CP	**43,500 IU**	86.67^{a}	94.48^{abc}	91.80^{ab}	82.86^{a}	88.31^{ab}	85.78^{ab}	93.42^{ab}	88.42^{ab}	87.77^{ab}	86.80^{ab}
48% CP	**87,000 IU**	85.45^{abc}	95.59^{a}	93.52^{a}	85.13^{a}	90.22^{a}	88.13^{a}	94.85^{a}	90.71^{a}	89.71^{a}	89.35^{a}
44% CP	**0 IU**	85.00^{abc}	95.21^{ab}	92.57^{a}	83.52^{a}	88.87^{ab}	85.96^{ab}	93.64^{ab}	88.60^{ab}	87.80^{ab}	86.96^{ab}
44% CP	**21,750 IU**	85.78^{ab}	93.67^{abc}	91.55^{ab}	82.98^{a}	90.33^{a}	85.69^{ab}	92.93^{abc}	88.70^{a}	87.84^{ab}	87.40^{a}
44% CP	**43,500 IU**	84.40^{abc}	93.49^{abc}	91.46^{ab}	82.54^{ab}	88.99^{ab}	85.84^{ab}	93.61^{ab}	88.24^{ab}	87.56^{ab}	87.03^{ab}
44% CP	**87,000 IU**	81.78^{c}	92.38^{c}	88.41^{b}	78.15^{bc}	86.65^{b}	82.23^{bc}	91.10^{bc}	85.33^{bc}	85.38^{bc}	83.51^{bc}
Standard Error		0.90	0.60	0.85	1.00	0.70	0.90	0.68	0.74	0.77	0.81
P-value		<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
Main effects											
SBM type	**48% CP**	84.90	94.41	91.36	82.15	88.53	85.27	93.00	87.98	87.24	86.43
	44% CP	84.24	93.69	91.00	81.80	88.71	84.93	92.82	87.72	87.14	86.22
Enzyme level			93.93				83.77	92.00	86.54^{b}	85.81^{b}	84.92^{b}
	0 IU	83.86		90.51	80.78	87.94					
	21,750 IU	85.27	94.29	91.61	82.77	89.45	85.64	93.14	88.50^{a}	87.74^{a}	87.04^{a}
	43,500 IU	85.54	93.98	91.63	82.70	88.65	85.81	93.52	88.33^{a}	87.66^{a}	86.91^{a}
	87,000 IU	83.61	93.98	90.97	81.64	88.43	85.18	92.98	88.02^{a}	87.55^{a}	86.43^{a}
Variation (P-value)											
SBM type (T)		0.273	0.097	0.547	0.626	0.713	0.590	0.711	0.617	0.866	0.722
Enzyme (E)		0.057	0.930	0.485	0.160	0.191	0.107	0.157	0.041	0.046	0.043
T × E		<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01

[1]Values are the least-square means of 8 replicate pens per treatment, each pen with 20 birds.
[2]Soybean meal 44% was formulated by adding soyhulls at a ratio of 12%, refer to Table 6.3.
[a-d]Means within a column not sharing a common superscript differ significantly (P < 0.05).

Table 6.13. Effects of SBM type and β-mannanase addition on non-essential AA digestibility of male broilers at 42 d of age[1]

SBM type[2]	Enzyme level	Pro, %	Tyr, %	Gly, %	Ser, %	Cys, %	Asp, %	Glu, %
48% CP	**0 IU**	83.57^{b}	82.11^{c}	80.18^{b}	82.17^{b}	74.19^{bc}	82.70^{b}	87.03^{c}
48% CP	**21,750 IU**	86.19^{ab}	86.13^{ab}	84.34^{a}	86.04^{a}	80.29^{a}	86.32^{a}	90.19^{ab}
48% CP	**43,500 IU**	86.45^{ab}	87.08^{a}	84.64^{a}	86.25^{a}	81.12^{a}	86.70^{a}	90.38^{a}
48% CP	**87,000 IU**	88.06^{a}	88.37^{a}	86.78^{a}	88.44^{a}	81.98^{a}	89.28^{a}	92.37^{a}
44% CP	**0 IU**	86.45^{ab}	86.64^{a}	84.75^{a}	86.42^{a}	80.84^{a}	86.87^{a}	90.15^{ab}
44% CP	**21,750 IU**	86.49^{ab}	85.40^{abc}	84.19^{a}	85.90^{a}	79.77^{a}	86.91^{a}	90.41^{ab}
44% CP	**43,500 IU**	86.35^{ab}	85.84^{abc}	84.27^{a}	86.10^{a}	78.76^{ab}	87.05^{a}	90.49^{ab}
44% CP	**87,000 IU**	83.67^{b}	82.35^{bc}	79.38^{b}	81.94^{b}	72.96^{c}	82.88^{b}	88.38^{bc}
Standard Error		0.73	0.90	0.87	0.81	1.10	0.74	0.62
P-value		<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
Main effects								
SBM type	**48% CP**	86.07	85.92	83.99	85.73	79.40	86.25	90.00
	44% CP	85.74	85.06	83.15	85.09	78.08	85.92	89.86
Enzyme		85.00	84.38				84.79^{b}	88.60^{b}
level	**0 IU**			82.47	84.30	77.51^{b}		
	21,750 IU	86.34	85.77	84.27	85.97	80.03^{a}	86.61^{ab}	90.30^{a}
	43,500 IU	86.40	86.46	84.45	86.17	79.94^{a}	86.87^{a}	90.44^{a}
	87,000 IU	85.86	85.36	83.08	85.19	77.47^{b}	86.08^{ab}	90.38^{a}
Variation (P-value)								
SBM type (T)		0.529	0.176	0.178	0.2751	0.085	0.537	0.757
Enzyme (E)		0.209	0.138	0.076	0.097	0.017	0.030	<0.01
T × E		<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01

[1]Values are the least-square means of 8 replicate pens per treatment, each pen with 20 birds.
[a-d]Means within a column not sharing a common superscript differ significantly (P < 0.05).

CHAPTER 7

CONCLUSIONS

The correct use of dietary fibers and fibrous ingredients continue to challenge poultry nutritionists worldwide as we seek to maximize nutrient utilization efficiency and subsequently, growth performance. Although it has been proposed that dietary fibers are associated with several negative effects when used in poultry diets, their ability to positively modulate digestive organ growth, intestinal morphology, nutrient digestibility and growth, when used in adequate amounts, supposes a practical way to improve efficiency and promote the utilization of fibrous by-products.

Chapter 3 (experiment 1) tested the theory that differences in growth performance when using dietary fiber are driven by fiber type and inclusion level. Two fiber types (i.e. soyhulls and cellulose) were compared in terms of growth performance, intestinal histomorphology, and nutrient digestibility. Addition of soybean hulls to the diets at 6% (4% CF) improved growth performance, intestinal morphology, and nutrient digestibility parameters. However, higher inclusions resulted in an abrupt decrease in performance and nutrient digestibility. This was not seen in treatments containing cellulose. Thus, the differences were attributed to differences in fiber types in natural feedstuffs. Therefore, chapter 4 attempted to replicate different fiber ratios using semi-purified diets with 0% crude fiber to assess the impact of soluble (pectin) to insoluble (cellulose) fiber ratios on intestinal parameters, viscosity, and nutrient digestibility and how those parameters affect growth performance. In this experiment, it was clear that high soluble/insoluble fiber ratios have a detrimental effect on intestinal development and nutrient digestibility that leads to reduction on growth performance. Most of the negative effects when

using soluble fibers were attributed to the ability to generate viscosity and reduce nutrient digestibility by interfering with normal nutrient metabolism. Trying to understand better the role of other physical characteristics of the dietary fibers, chapter 5 investigated the effect of changing the physical structure (i.e. particle size) of dietary fiber in two different fiber types and inclusion levels. In this experiment, it was clear that particle size plays a critical role in the stimulation of the upper gastrointestinal tract (i.e. gizzard) and it should be accounted for whenever using fibrous ingredients. Furthermore, results from this experiment indicated that upregulation of nutrient transporters are not necessarily associated with increases in nutrient digestibility but might be a way to offset the lower nutrient uptake at the enterocyte level when feeding high fiber level and, therefore, such changes in nutrient digestibility should always be linked together to more stable parameters such as weight gain and feed efficiency when trying to determine the positive or negative effects of fibrous ingredients. The experiments from chapter 3 to chapter 5 were conducted in battery cages where birds only had access to the fiber contained in the dietary treatment and evaluation of dietary fiber is more feasible. Furthermore, diets had been provided as mash without any other physical alteration (i.e. pelleting). Therefore, chapter 6 attempted to integrate results from previous chapters by formulating real-world pelleted diets and conducting an experiment in floor pens using dehulled, 48% SBM versus soyhulls-added 44% SBM. The additions of soyhulls to diets had numerical improvements in growth performance and also had the best results in terms of intestinal morphology parameters. The fact that birds had access to additional fiber source (i.e. wood shavings), could not be circumvented. However, as was previously seen in chapter 3, the inclusion of low levels of soyhulls (3%) had similar or better results in weight gain and feed efficiency when compared to the control group.

Collectively, these results indicated that soybean hulls can be added to broiler diets at levels as high as 6% in diets of broiler chickens and that dietary fibers from soyhulls can be strategically used as functional nutrient to positively modulate intestinal development, nutrient digestibility, and subsequently, growth performance. Future research should focus on elucidating the impact of physiological changes in organ growth exerted by dietary fibers in terms of energy requirements and expenditure for maintenance and growth and investigate individual fibrous ingredients and determine their potential to be integrated in poultry diets. This might provide us a pathway through which we may be able to reduce production costs and improve production efficiency.