The role of feed processing and fiber addition on improving the nutrition and growth performance of broilers

by

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B.S., University of Cape Coast, 2004
M.Phil., University of Ghana, 2014

AN ABSTRACT OF A DISSERTATION

submitted in partial fulfillment of the requirements for the degree

DOCTOR OF PHILOSOPHY

Department of Grain Science and Industry
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Abstract

Three experiments were conducted to study the growth performance, relative gizzard weights, intestinal morphology, and blood serum parameters response of broiler chickens fed crumbled pellets, mash diets, and diets containing increasing levels of wheat bran with or without exogenous enzyme supplementation.

In Experiment 1, a common corn-soybean meal (corn-SBM) diet was formulated to meet or exceed the nutritional requirements of Cobb 500 broiler starter, grower, and finisher birds and fed to 900 chicks raised in floor pens. Dietary treatments were fed to only starter chicks to evaluate the impact of starter feed form on subsequent growth performance of broiler chickens and comprised of 1) mash diet, 2) 7 d crumbles followed by 14 d mash, 3) 14 d crumbles followed by 7 d mash, and 4) 21 d crumbles. Results indicated that feeding crumbles increased ($P < 0.001$) BW, ADG, ADFI, and improved ($P < 0.001$) FCR of broilers compared to feeding the mash diet.

In Experiment 2, a study was conducted to determine the effects of crumble size and quality on broiler performance. A common corn-SBM diet was formulated and fed to 300 Cobb 500 broiler chicks raised in battery cages for 21 d. Broilers were fed a mash diet (M), micro-pellets (MP), and coarse- or fine-crumbles with or without the fines removed. Results showed that feeding MP or crumbles resulted in increased ($P < 0.001$) BW, ADG, ADFI, and improved ($P < 0.001$) FCR of broilers compared to feeding the M diet. Removing the fines from the crumbles further improved the BW and FCR of broilers fed the crumbled diets. Additionally, feeding M increased the relative gizzard weight of broilers compared to those fed MP or crumbles.

In Experiment 3, the growth performance, intestinal morphometry, and blood serum parameters of broilers fed diets containing increasing levels of wheat bran (WB) and supplemented with exogenous multi-enzyme containing Xylanase, β-Glucanase, and Phytase (XGP) was evaluated in a 42-d trial. Four basal diets were formulated to meet or exceed the nutritional requirements of Cobb 500 broiler starter, grower, and finisher birds. A common starter diet was fed to all day-old chicks for 7 d with treatment diets fed to grower and finisher birds only. A total of 720 seven-day-old broilers were raised in 48 floor pens and fed diets with or without supplemental XGP. The addition of XGP had no effects on BW, ADFI, ADG, and FCR. Supplementation of the 12% WB with XGP resulted in similar performance as the 0% WB diets. The addition of both the WB and XGP increased the villi height, villi height: crypt depth but had no influence on crypt depth. Additionally, XGP increased ($P < 0.01$) high density lipoprotein-cholesterol, but decreased red blood cells, hemoglobin, and cholesterol concentration of the blood.

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Approved by:

Major Professor
Dr. Charles R. Stark

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Dedication

I dedicate this work to the memories of my late mother, Madam Dorothy Saah, who unfortunately couldn’t live long enough to see the completion of my Ph.D. studies at Kansas State University. I also dedicate this to my dearest wife, Ernestina Wiafe Idan, and my daughters, Anaya Maame Efua Idan, Adriel Ewura Efe Idan, and Ariana Ewura Abena Idan, for their unflinching support throughout my graduate school days.

Preface

This dissertation is made up of six chapters. The first two chapters provide the background and the review of the literature to the study. The references for the first two chapters are combined. Chapters Three and Four were written per the guidelines of the Journal of Applied Poultry Research, whereas chapter Five was based on the guidelines of the Poultry Science Journal. Chapter Six provides information about the key findings from the research leading to the completion of the current dissertation.

Chapter 1 - Introduction

Feed cost accounts for 60 to 70% of the total variable cost of broiler production (Swain, 2017) with the cost of ingredients making up the major portion of the feed cost (Behnke and Beyer, 2002). Pelleting broiler diets increases the cost of the diet, but the additional cost is normally offset by improved broiler performance (Behnke and Beyer, 2002; Nolan et al., 2010).

Feed processing involves several operations, including receiving, grinding, mixing, batching, packaging, warehousing, and loadouts. Pelleting is an optional step that is used in most developed countries while most developing countries rely on feeding mash diets. The manufacturing process must be done correctly to ensure optimal animal performance (Behnke and Beyer, 2002). Manufacturing quality feed uses a variety of processes, which include particle size reduction, proportioning and mixing, as well as thermal processing such as pelleting, extrusion, or expansion (Schofield, 2005; Goodarzi et al., 2016). Each of these technologies add to the cost of feed and thus should provide a return on the investment in broiler performance. Mash feed is less expensive because it involves less processing steps and lower energy inputs compared to pellets/crumbles. However, research has indicated that mash feed is less palatable to broilers compared to pellets, thus reducing feed intake (Jahan et al., 2006). Therefore, most broiler production systems in the USA feed crumbles and pellets. The pelleting process is commonly used in the production of broiler feed and is described as “the agglomeration of small particles into larger particles utilizing a mechanical process in combination with moisture, heat, and pressure (Falk, 1985).

Feeding pellets to broilers stimulates feed intake resulting in higher body weight and improved feed conversion compared to mash diets (Amerah et al., 2008; and Chewing et al., 2012). The improved performance may be attributed to decreased feed wastage, reduced selective feeding, increased palatability and digestibility of feed, decreased ingredient segregation, reduction of time and energy spent during prehension, thermal modification of starch and protein, and destruction of pathogenic organisms (Behnke 1994; 1998). In addition, Ghazi et al., (2012) suggested that pelleting increased the bulk density of feed, improved feed flowability, and provided the opportunity for the addition of alternative ingredients.

Broilers are often fed alternative feed ingredients to reduce the cost of the feed, which decreases total production cost. Alternative ingredients are typically sourced from either ethanol plants or food processing plants. Cereal co-products such as wheat bran, middlings, shorts, oat hulls, rice hulls, and rice bran make up the majority of the by-products commonly incorporated into poultry diets. However, the utilization of these ingredients is limited due to the presence of non-starch polysaccharides (NSP) and fiber (NSP plus lignin) (Choct and Annison, 1990), which reduces feed intake, and growth performance. The inclusion of fiber in moderate amounts up to 4% has been reported to improve the growth performance of broilers by stimulating gizzard activity to increase digestion of mash-based diets (Jiménez-Moreno et al., 2009b; Gonz´alez-Alvarado et al., 2010). However, high levels of fiber can encapsulate potentially available nutrients, decreasing their digestibility, and impairing growth performance of broilers (Bach Knudsen, 2014). Soluble NSPs have viscous properties which may interfere with the digestion process thus decreasing the digestibility of other nutrients (Steenfeldt, 2001).

Broilers and other monogastric animals do not produce endogenous enzymes that are capable of breaking down NSPs and fiber. Therefore, exogenous carbohydrases have been added to diets that contain relatively higher levels of fiber to increase the digestibility of nutrients for poultry and improve growth performance (Bedford, 2000). The supplementation of diets high in fiber with carbohydrases reduces the antinutritive effects of NSPs, improves feed utilization efficiency, increases growth rate, improves gastrointestinal tract health, and reduces environmental pollution due to a decreased output of manure and gases such as ammonia (Bedford, 1997; Choct, 1997; Marquardt, 1997).

Objectives of the Study

The objectives of the research conducted and presented in this dissertation were:

1. To investigate the effects of feeding crumbles to broiler chicks for the first 21 d post-hatch compared to feeding a mash diet for a designated period on subsequent growth performance.
2. To compare the growth performance, feed conversion ratio, and relative gizzard weight of broiler chicks fed diets in the form of mash, micro pellets, or coarse and fine crumbles either with or without the fines removed.
3. To investigate the growth performance, hematological and serum blood parameters, and villi height and crypt depth of broilers fed diets containing increasing levels of wheat bran with or without multi-enzyme supplementation.

Chapter 2 - Literature Review

Processing Feed for Broiler Production

Feeds manufactured for animals including broilers undergo several operations from receiving, grinding, mixing, batching, thermal treatments (pelleting, extrusion, and expansion), packaging, warehousing to loadouts. According to Behnke and Beyer (2002), each of these operations has the potential of influencing feed quality positively or negatively, which subsequently affects animal performance.

The feed should supply the daily nutrients requirements for the bird and promote growth. The bird's potential to attain its daily nutritional requirements depend on 1) the nutrient composition of the diet and 2) feed or nutrient intake. Nonetheless, the performance of birds is related to feed intake. For this reason, increased feed intake should result in more efficient broiler growth and nutrient utilization. Feed intake and growth performance can be significantly influenced by physical feed form (Dozier et al., 2010). The three physical forms of feed that play an important role in broiler growth performance are mash, pellets, and crumbles (Fig. 2.1). Increased feed intake is very important to ensuring efficient nutrient utilization and proper growth of broilers. Thus, feeding mash diets or pellets/crumbles containing more fine particles tend to reduce feed intake and negatively affect growth rate and animal performance.

Mash Diet

Mash is a complete feed manufactured with ground cereal grains and processed ingredients that are mixed such that each mouthful provides a well-balanced diet (Agah and Norollahi, 2008). The size and uniformity of particles determine the quality of the mash diets, hence animal performance is expected to improve with diets containing more uniform particles.

Abdollahi et al. (2013) reported that feeding a mash diet is less expensive to produce, encourages uniform growth, and leads to less death losses. However, according to Jahan et al. (2006), ground feed is not as palatable as compared to coarse grains resulting in lower feed intake. Briggs et al. (1999) reported the poor flowability of mash diets in mechanical feeding systems led to poorer growth performance for meat birds. Additionally, the flowability of mash diets on broiler performance depends on the grinding equipment. Comparatively, roller mills produce more uniform particle sizes than hammermills in which particle sizes tend to be more variable with a greater number of fine particles (Reece et al., 1985). Uniformity of particle size is very important in determining the effects of mash diets on bird performance as less time is spent in selecting larger particles. According to Gentle (1979) birds can use their mechanoreceptors located in the beak to distinguish between different particle sizes in feed. While some authors reported that mill type influences bird performance, Nir et al. (1990) found no effects on performance when diets of similar geometric mean particle diameter were ground with a hammermill or roller mill.

It is important to note that feed particle sizes are what birds perceive and touch. Schiffman (1968) reported that birds prefer larger feed particles; which is preferred at all ages (Portella et al., 1988); and as they advance in age, the preference for larger particle size increases (Nir et al., 1994b). Birds' performance and digestive tract morphology is also reported to be linked with the consumption of diets with different particle sizes (Nir, 1998). This may be attributed to the width of the birds' beak (Amerah et al., 2007). Amerah et al. (2007) suggested that finer grinding increases substrate availability for enzymatic digestion. However, there is evidence that coarser grinding to a more uniform particles size improves the performance of birds fed mash diets.

Figure 2.1. Different feed forms used in broiler production.

The Pelleting Process

The pelleting process is described by Falk (1985) as “the agglomeration of small particles into larger particles by means of a mechanical process in combination with moisture, heat, and pressure”. Thus, pellets are formed by passing conditioned mash through a metal die and subsequently into a cooler. Depending on the purpose of production, pellets are fed intact or crumbled by passing them through a single pair roller mill. The pelleting process has become a major component of modern feed manufacturing technology because the cost of pelleting is offset by improved animal performance (Behnke, 1998). It is generally recognized that feeding pelleted diets improves feed intake and growth rate of broilers (Calet, 1965; Choi et al., 1986; Nir et al., 1994b). However, good quality pellets must be fed to optimize growth performance of birds (Briggs et al., 1999). Fairfield (2003) suggested that feed particle size, condition temperature, moisture content of the conditioned mash, die specifications, and cooling are among the major factors that affect pellet quality. Feeding quality pellets improves feed efficiency, growth, and uniformity of broilers (Keith and Behnke, 2001).

Pellet Crumbling

Broiler chicks are unable to fully utilize pelleted diets due to the size of their beak. Therefore, it is important to provide them with smaller pellets consistent with the width of their beaks. Moreover, since producing pellets through very small die holes is not economical, lager pellets are produced and crumbled to produce a smaller feed particle size, which are coarser than mash. Crumbling is done by shearing whole pellets using a specially designed single pair roller mill (Fairfield et al., 2005). The pellet crumbler operates with a pair of rolls that turn at the same speed or with a small differential speed with one roll turning slightly faster than the second roll. According to Fairfield et al. (2005), size of the crumbles produced depends on the roll gap (space between the rolls) and corrugation (groves in the roll surfaces). Feeding crumbled diets gained popularity in broiler production due to its convenience of feeding (Reece et al., 1985). Higher feed consumption has also been reported to be associated with feeding starter chicks crumbled diets (Choi et al., 1986).

Effects of Pelleting on Broiler Performance

Feeding pellets is generally believed to improve broiler growth performance and feed efficiency. These improvements are due to increased feed intake; decreased feed wastage; decreased ingredient segregation, less time and energy spent on prehension, reduced selective feeding, destruction of pathogenic organisms, and increased nutrient digestibility (Behnke, 1994). Equally important, pelleting increases the bulk density of feed, improves feed flowability, and provides opportunities for reducing formula costs by incorporating alternative feed ingredients into the diets. While these benefits are important, pelleting must be cost-effective. A commercial feed mill must have enough added margin to cover the operational cost of producing the crumbles or pellets. In the case of integrated feed mills, improved feed conversion must offset the cost of pelleting (Fairfield, 2003). Nonetheless, pelleted diets are the most widely used form of feed in poultry and swine production.

The physical form of feed is thought to significantly impact feed intake and feeding pellets has been identified as a strategy to increase feed intake (Dozier et al., 2010). Feeding pelleted diets also reduces the presence of smaller particles in the feed thus increasing feed intake (Peisker, 1994). Furthermore, Bolton and Blair (1977) reported that a 10% increase in feed intake in broilers fed crumbles or pellets compared to mash. Dozier et al. (2010) observed that broilers fed higher quality pellets had higher feed intake than those fed a mash diet during a 42-day study. The authors reported a 5.6% higher feed intake for the birds fed high quality pellets as compared to those fed mash diets, which resulted in a higher BW. Similarly, Choi et al. (1986) reported that birds fed pelleted diets consumed 10% more feed and were 11% heavier than those fed mash diets from 28 to 56 d of age. Abdollahi et al. (2013) reported feeding broiler chickens pellets or crumbles improve broiler performance by increasing feed intake, which results in improved growth performance and feed conversion ratio. The effect of feeding crumbles and pellet quality on broiler performance and gizzard weight was investigated by (Hu et al, 2012). The authors demonstrated that feed intake increased in response to feeding crumbles and quality pellets, which resulted in heavier BW compared to feeding a mash diet. In addition, a higher feed intake of between 7 to 10% was reported for broilers fed crumbles compared to those fed mash diets (Zang et al., 2009).

In a more recent study, Massuquetto et al. (2019) evaluated the effect of different feeding programs and pelleting on performance, nutrient digestibility, ileal digestible energy (IDE); and carcass yield of broilers from 21 to 35 d of age. The authors fed mash and pelleted diets on an *ad libitum* basis, or pelleted diet provided at (100%) or restricted at 95, 90, and 85% of the amount consumed by the birds fed the mash diet. They observed that feeding broilers pelleted diet *ad libitum*, resulted in the higher feed intake (11%) and weight gain (17%), and improved FCR (6%) as compared to feeding the mash diet. The authors suggested the improved broiler performance was a result of increased feed intake. Lemons et al. (2019) conducted a study to investigate the interactive effects of feed form and phase of feeding on performance of Ross 708 male broilers throughout a 46 d grow-out period. The authors reported that broilers fed crumbles (1785µm) had increased feed intake, improved FCR, and a 9 g improvement in BWG at 14 d compared to broilers fed ground crumbles (988 µm). The authors suggested that increased feed intake was due to better feed integrity observed in the broilers fed the crumbled diet, which resulted in an improvement in performance (BW and FCR) compared to those fed the ground crumbles or the mash diet.

Feed wastage is reduced when birds are fed pellets (Behnke, 1994). Feed wastage in broilers is partly attributed to the avian anatomy. With no teeth, broilers and turkeys generally cannot easily grasp food. Smaller particles tend to fall from the birds' mouth especially when fed diets with variable particle sizes. However, during pelleting, smaller particles are agglomerated into larger ones preventing small particles from falling from the mouth of the birds (Behnke and Beyer, 2002). Additionally, feeding pelleted diets prevents selective feeding which is observed in mash diets and contributes to feed wastage. Jensen (2000) reported that pelleting broiler diets reduced feed wastage due to less particles from falling from the beak onto the floor or into the water. In addition, providing diets in the pelleted form prevents birds from selecting larger particles from mash diets. Sorting due to selective feeding pushes feed out of feeders thus increasing feed wastage (Abdollahi et al., 2013). Pelleting also reduces segregation of ingredients such as limestone during the feed manufacturing process thus keeping them fixed in the pellets (Greenwood and Beyer, 2003). Several researches have shown that birds prefer feed with larger particles than smaller ones. Thus, when presented with feed of different particle sizes as can be found in mash, they tend to select the larger particles. To prevent this selective feeding birds are generally fed pelleted diets. Feeding pelleted diets ensures that each animal receives a balanced of nutrients by preventing the animals from picking and choosing between ingredients. In a study conducted by Scheideler (1991), birds fed a diet that contained pellets and fines and the pellets were consumed first.

Birds consuming mash diets spend more time standing at the feeder to consume feed to fill the crop. This results in poor feed conversion efficiency since more energy is expended during feeding. On the other hand, birds fed pelleted diets spend less time and energy ingesting the feed thus consuming more nutrients per every unit of energy expended (Scheideler, 1991; Jones et al., 1995). Furthermore, Jensen et al. (1962) recorded the time spent by broiler chickens consuming feed during a 12-hour period from 21 to 28 d and observed that birds fed pellets spent 4.7% vs 14.3% of the time at the feeder compared to those fed the mash diet. In a like manner, Nir et al. (1994b) observed that birds fed pellets were less active and spent less time eating feed than those on mash diet from 28 to 40 d. Modern commercial birds are grown to heavier weights hence are unable to stand for long periods of time, thus feeding pellets ensures optimal growth since less time is spent consuming feed.

The thermal treatment of mash during the pelleting process has been reported to improve nutrient digestibility unless the treatment is too severe. According to Zimonja et al. (2008) only a small amount of starch gelatinization has been attributed to the pelleting process, indicating a modest importance of pelleting to starch digestion (Svihus et al., 2004; Zimonja et al., 2008). However, conflicting reports on the effects of pelleting on nutrient digestibility has been reported. López et al. (2007) evaluated the effects of different feed forms (mash, pellets, and expanded pellets) on broiler performance. The authors observed no differences in the coefficient of total tract apparent digestibility of dry matter (DM), crude protein (CP), and crude fiber (CF) among the processed diets. However, as compared to birds on mash diet, the coefficient of total tract apparent digestibility of CP, CF, and apparent metabolizable energy (AME) in processed diets were improved significantly. The improvement in the coefficient of total tract apparent digestibility of CP, CF, and AME of the pelleted feeds was attributed to the temperature, moisture, and pressure used during the processing that dissolved the grain cell walls facilitating access of digestive enzymes to the cell contents. Starch gelatinization is reported to occur at conditioning temperatures ranging from 45 to 95ºC depending on the type of starch and moisture. According to Rooney and Pflugfelder (1986), gelatinization opens up starch granules thus increasing starch digestibility by enzymes. Pelleting can also increase starch digestibility by denaturing α-amylase inhibitors as a result of the exposure of mash diets to moisture, pressure, and temperature. Saunders (1975) reported that the level of α-amylase inhibitors in wheat bran was reduced by pelleting which increased the digestibility of starch. However, severe thermal treatment for a prolonged time could rapture glycosidic linkages in diets' carbohydrates thus decreasing starch digestibility (Hoover and Vasanthan, 1994). The physical structure of proteins changes when exposed to thermal treatment during pelleting resulting in changes in the reactivity, functional, and nutritional properties (Voragen et al., 1995). According to Camire et al. (1990) pelleting with moderate heat could improve protein digestibility by inactivating enzyme inhibitors as well as denaturing protein thus exposing new sites for enzymatic action. Moreover, severe thermal treatment could result in a Maillard reaction of some feed constituents leading to decreased availability of lysine.

Wheat By-product Ingredients in Poultry Nutrition

The two major ingredients used in the formulation of poultry diets are corn and soybean meal. The high demand of corn for human consumption and ethanol production, has resulted in the steady rise of corn prices over the past few years. The search for alternative fibrous ingredients (AFI) and how to effectively utilize them in poultry diets has become increasingly important due to drought and other competing interests for traditional ingredients. The concept of AFI involves the inclusion of a relatively small to medium amount of fibrous ingredients into poultry diets without negatively impacting performance. Wheat by-products from the flour milling process are commonly used for poultry and livestock feeds. Partial replacement of corn and SBM with wheat by-products will reduce the total cost of poultry production. The major wheat by-products available for use in poultry diets are wheat bran, wheat shorts or brown shorts, wheat screenings, and wheat middlings (Patrick and Schaible, 1981). Among these by-products, wheat bran and wheat middlings have been the most extensively studied and utilized in animal feed formulations because they have very little human food value hence, they have a greater potential to reduce poultry feed costs (Dale, 1996; Ahmadi and Karimov, 2010).

Wheat bran (WB) is a by-product of dry milling of wheat to produce flour and consists of the outer layers (cuticle, pericarp, and seed coat) combined with small amounts of starchy endosperm of the wheat kernel (Hemery et al., 2007). Wheat bran is composed of 50% of wheat offals and about 10 to 19% of the kernel, depending on the variety and milling process (Ash, 1992; Hassan et al., 2008). WB contains relatively high crude protein (15-17%), phosphorus and magnesium, but has low metabolizable energy (ME), calcium, and starch content (NRC, 1994). Heuzé et al. (2015) reported the chemical composition of WB used in the formulation of poultry diets (Table 2.2). The incorporation of WB at varying amounts into poultry diets will reduce the amount of corn and ultimately feed cost. However, the higher fiber and lower ME contents of WB indicates that poultry must consume more feed to meet their energy requirements as compared to corn.

Wheat middlings (WM) is another popular wheat by-product that is used as an energy source in poultry diets. Wheat middlings are not typically used as a human food source, which makes them more available at a lower cost. In the US, the most common by-products from the commercial milling of wheat is WM and consists of fine particles of wheat bran, wheat shorts, wheat germ, wheat flour, starchy endosperm, and some of the offal from the “tail end of the mill” (AAFCO, 2015). It is the end-product of the roller milling of wheat grain to remove the bran and the grinding of the endosperm. Compared to the wheat and corn grains, WM contains a much lower ME, digestible energy (DE), and net energy (NE) but relatively higher CP and CF content. Wheat screenings is a wheat by-product obtained after the cleaning and grading of wheat and is composed of thin, broken and shrunken wheat kernels, weed seeds and other contaminants including straw, chaff, and dust (Slominski et al., 2004). AAFCO (2015) described the other wheat by-products as: 1) Wheat Red Dog consists of the offal from the “tail of the mill” together with some fine particles of wheat bran, wheat germ, and wheat flour containing less than 4% CF. 2) Wheat Mill Run is made up of coarse and fine particles of wheat bran, wheat shorts, wheat germ, wheat flour, and the offal from the “tail of the mill containing less than 9.5% CF. 3) Wheat Shorts comprised of fine particles of wheat bran, wheat germ, wheat flour and the offal from the “tail of the mill.”

These wheat by-products are reported to be extremely variable in composition and are therefore recommended to be used in moderate inclusion rates in diets of young poultry up to 10% (Leeson and Summers, 1991). However, a common characteristic of all wheat by-products is the presence of higher non-starch polysaccharides (NSP) and fiber (NSP plus lignin) content (Bach Knudsen, 1997; Adeola and Cowieson, 2011). Dietary fiber (DF) consists of a very diverse group of polymers with varying physicochemical properties, thus hampering the prediction of the nutrient composition of the fiber fractions. In poultry nutrition, the term DF was first coined by Hipsley in 1953 (DeVries et al., 1999) to describe the non-digestible components of plant cell wall. According to AACC (2001) DF is made up of the carbohydrate portion of the plant cell wall, which are indigestible by the endogenous enzymes of monogastric animals including poultry. The most extensively studied components of plant cell materials are NSPs because of their role in depressing digestion in the poultry. These heterogeneous group of compounds have unique physicochemical properties with varying degrees of water dissolution, size, and structure (Zargi, 2018). In poultry nutrition, NSPs include cellulose, hemicellulose, pentosans (D-glucans, mannans, arabinans and galactans, and galactomannan, xyloglucans) and pectic polysaccharides (polygalacturonans, and rhamnogalacturonans) and theses are reported to have negative effects on performance when present in large quantities (Adeola and Cowieson, 2011).

Bailey (1973) classified NSPs into three main groups namely cellulose, non-cellulosic polysaccharides (NCP) and pectic polymers/ polysaccharides (PP) (Table 2.1). These groups of NSPs have different levels of solubility and are further categorized as soluble or insoluble. Soluble NSPs are partially soluble in water, alkali or dilute acids and include mostly NCP and PP. On the other hand, insoluble NSPs are comprised mainly of cellulose, hemicellulose, and lignin. The reported growth performance of poultry has varied based on the inclusion level of soluble and insoluble NSPs. For instance, soluble NSPs, such as arabinose, inulin, pectin, gum, and beta-glucans are reported to increase digesta viscosity of the intestine, increase the solubility and water holding capacity of the diet, limit the gizzard capacity as well as increase the digesta retention time (Jimenez-Moreno et al., 2010; Mateos et al., 2013). Increased digesta viscosity in the small intestine has been reported to reduce feed intake and growth performance of birds (Mateos et al., 2013). On the contrary, a moderate amount of insoluble fiber is reported to have a positive effect on gizzard activity and development, and GIT reflux, thus promoting nutrient digestibility (Hetland et al., 2004).

Different analytical methods have been used for the analysis of the fiber content in poultry feeds. Nutritionists need to know the fiber content of the WB in order to determine the quantity of WB to include in the diet as well as the type of exogenous enzymes to include to increase the digestibility of diets. The approved analytical methods for WB include the crude fiber method (Henneberg and Stohmann, 1859), the detergent method (Van Soest, 1963, 1984; Van Soest and Wine, 1967), enzymatic-gravimetric AOAC (Association of Official Analytical Chemists) and enzymatic-chemical Englyst (Englyst et al., 1994) and Uppsala procedures (Theander et al., 1994). The CF method is the oldest analytical procedure for estimating fiber and is still used in the proximate analysis of feeds. The CF categorizes feed carbohydrates into a more digestible component, "nitrogen free extract (NFE)" and a less digestible fibrous component, CF. The CF method has an added advantage of separating feed into various components (NFE, CF, moisture, ash, ether extract, i.e., crude fat, and crude protein). However, values obtained are lower compared to the other analytical procedures because none of the soluble NSPs are accounted for (Bach Knudsen et al., 2013).

The detergent method was originally developed for the analysis of fiber rich feedstuffs but is currently used in the analysis of all feeds/ingredients including concentrated feeds (Bach Knudsen, 2014). The procedure involves treating feed samples with a either acid or neutral buffered detergent, which separates the fiber components into Neutral Detergent Fiber (NDF) and Acid Detergent Fiber (ADF). According to Choct (2002) NDF measures the insoluble portion of the NSP plus lignin, whereas ADF determines a portion of insoluble NSP comprised mainly of cellulose and lignin. The hemicellulose component covers a variety of NSPs including arabinoxylans, mixed linked ß-glucans, xyloglucans, mannans, galactomannans, galactans, arabinans and other cellulolytic polysaccharides. Hemicellulose is, therefore, determined using the equation below:

NDF – ADF = Hemicellulose.

The enzymatic-gravimetric methods utilize enzymatic removal of non-cell wall organic materials and gravimetrically measures the residue corrected for ash. The enzymatic-chemical method of fiber analysis is summarized by Bach Knudsen (1997) using the flow diagram in Figure 2.2. The Uppsala method quantifies individual sugar residue by converting them into alditol acetates and measuring them using a gas chromatograph (Theander et al.,1995). This method determines the lignin and uronic acids separately and is useful in the separation of the individual sugar composition of dietary fiber.

Wheat by-products utilized in the formulation of poultry diets contain varying levels of dietary fiber depending on the source. The inclusion of dietary fiber such as WB in poultry diets has traditionally been considered as a means of diluting the energy content which negatively impact feed intake and digestibility of nutrients (Rougiere and Carre, 2010; Navidshad et al., 2015). Most of the earlier research reports have associated poor growth performance and nutrient digestibility to the inclusion of DF in poultry diets (Jørgensen et al., 1996; Sklan et al., 2003). However, recent studies have indicated that the inclusion of moderate amounts of by-products containing dietary insoluble fiber could improve the growth performance and development of the digestive organs of broiler chickens by improving intestinal health (Gonzalez-Alvarado et al., 2007; Hetland and Svihus, 2007; Jiménez-Moreno et al., 2009b; González-Alvarado et al., 2010). The existing microbiota profile in the distal part of the GIT is also reported to be influenced by the amount and type of DF, and the composition of the basal diet. Li et al. (2019) investigated the effects of inulin and wheat bran only during the starter period or during the entire rearing life of broilers on growth performance, small intestine maturation, and cecal microbial colonization until slaughter. The authors fed broilers diets containing no inulin and no WB (CON), 2% inulin (IN), 10% wheat bran (WB), or 2% inulin + 10% wheat bran (IN+WB) to 11 d of age and reported that birds fed the WB/CON had increased BW and improved FCR compared to the other diets. They concluded that WB improved BW and FCR at the starter phase and continued even after supplementation was stopped indicating that including 10% WB in young broiler diets could be beneficial.

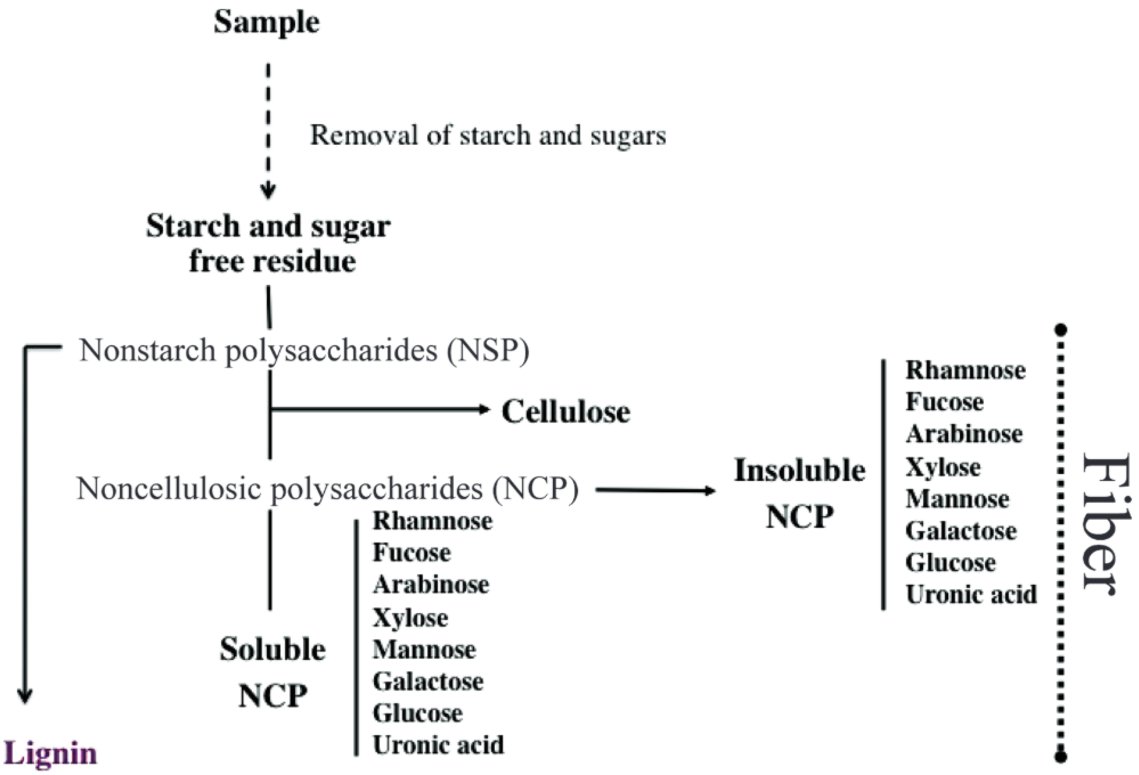

Figure 2.2. Determination of non-starch polysaccharides, lignin, and fiber by enzymatic-chemical procedure.

Source: Bach Knudsen (1997).

Table 2.1. Classification of NSPs

Category	**Monomeric residue**	**Linkage**	**Sources**
Cellulose	Glucose	β-(1→ 4)	Most cereals and legumes
Non-cellulosic polymers			
Arabinoxylans	Arabinose and Xylose	β-(1→ 4)-linked xylose units	Wheat, rye, barley, oat, rice, sorghum
Mixed-linked β-glucans	Glucose	β-(1→ 3) and β-(1→ 4)	Oat and barley
Mannans	Mannose	β-(1→ 4)	Coffee seed
Galactomannans	Galactose and mannans	β-(1→ 4)-linking mannan chain with α-(1→6) galactosyl side groups	Locust bean gum and guar gum
Glucomannans	Glucose and mannans	β-(1→ 4)-linked mannan chain with interspersed glucose residues in the main chain	Sugar-beet pulp, lilies, irises
Pectic Polysaccharides			
Arabinans	Arabinose	α-(1→5)	Cereal co-products
Galactans	Galactose	β-(1→ 4)	Sugar bean meal, sugar-beet pulp
Arabinogalactans (Type I)	Arabinose and Galactose	β-(1→ 4)-galactan backbone substituted with 5-linked and terminal arabinose residues	Grain legumes
Arabinogalactans (Type II)	Arabinose and Galactose	β-(1→ 3,6)-galactose polymers substituted with 3- or 5-linked arabinose residues	Rapeseed cotyledon

Source: Sinha et al. (2011)

Table 2.2. Average or predicted values of the composition of wheat bran

Main analysis	**Mean**	**SD**	**Min**	**Max**	**Number of samples**
Dry Matter, % as fed	87.0	1.1	83.6	90.3	19914
Crude Protein, % DM	17.3	1.0	14.1	20.5	19450
Crude Fiber, % DM	10.4	1.3	6.3	14.7	19300
NDF, % DM	45.2	4.6	32.4	56.5	743*
ADF, %DM	13.4	1.6	8.4	17.6	754*
Lignin, % DM	3.8	0.7	1.9	5.3	452*
Ether Extract, % DM	3.9	0.6	2.1	5.7	9117
Ash, % DM	5.6	0.5	4.0	7.3	11055
Starch (Polarimetry), % DM	23.1	4.0	11.1	35.4	17115
Total Sugars, % DM	7.2	1.6	3.7	10.5	152
Gross Energy, MJ/kg DM	18.9	0.3	18.0	19.9	65*
Minerals, g/kg DM					
Calcium	1.4	0.4	0.2	2.9	684
Phosphorus	11.1	1.2	7.8	14.6	890*
Potassium	13.7	2.4	8.3	18.4	81*
Sodium	0.1	0.1	0.0	0.3	128
Magnesium	4.5	1.1	2.2	7.0	66
Manganese	114	35	41	188	38
Zinc	89	19	55	136	37
Copper	13	4	2	21	40
Iron	155	45	58	253	26
Amino Acids, % Protein					
Alanine	4.6	0.3	3.8	5.2	25
Arginine	6.8	0.8	4.7	8.5	27
Cystine	2.1	0.2	1.6	2.5	39
Histidine	2.7	0.3	2.1	3.2	26
Isoleucine	3.2	0.2	2.6	3.6	28
Leucine	6.0	0.4	5.4	7.2	27
Lysine	4.0	0.4	3.1	4.9	70
Methionine	1.5	0.2	1.1	1.8	53
Phenylalanine	3.9	0.3	3.2	4.5	27
Threonine	3.2	0.2	2.9	3.5	27
Tryptophan	1.4	0.2	1.0	1.6	17
Poultry Nutritive Values, MJ/kg DM					
AMEn Cockerel	7.8	1.6	5.3	10.6	16*
AMEn Broiler	7.4		5.3	7.4	2*

* Average value obtained using an equation.

Source: Heuzé et al. (2015)

Feed Enzymes in Broiler Production

History of Enzyme Utilization

The use of feed enzymes in animal production is well recorded. In poultry production, the first report of an enzyme was Protozyme in the 1920s (Ewing, 1963). In the last 50 years, the use of enzymes in poultry feeding systems is considered as one of the major nutritional advances in animal nutrition. Nutritionists have long realized the benefits of including enzymes in poultry diets. However, the first reported use of commercial feed enzymes was in the United States in the 1950s and 1960s. Jensen et al., 1957 reported the importance of enzyme addition to barley-based diets. In the late 1960s, Nelson et al. (1968) and Rojas and Scott (1969) reported phytases for improved the availability of phosphorus (P) in diets formulated with plant-based proteins fed to chickens. However, the supplementation of wheat and barley diets in the 1980s with feed enzymes transformed the poultry industry in Europe. However, the enzyme preparations and products utilized in the early studies were considered to be crude and were available in only small quantities (Ravindran, 2013).

In the 1990s, the Netherlands, Germany, and the United States started to include phytase feed enzymes in pigs and poultry feed to reduce the P excretion to the environment (Bedford and Partridge, 2011). Since this time enzyme utilization in animal feeding systems has steadily increased. Freedonia (2009) reported an average annual growth rate of 13% between 1998 and 2008 for the animal feed enzyme market. Currently, the available feed enzymes used for poultry include phytases, proteases, and carbohydrases such as α-galactosidases, glucanases, xylanases, α-amylases, pectinases, and polygalacturonases (Chesson, 1993; Bhat, 2000; Adrio and Demain, 2014) with phytases having the largest market share.

Feed Enzymes in Poultry Nutrition

The utilization of feed enzymes in poultry diets has become increasingly important due to the release of energy and nutrients from otherwise indigestible ingredients. Poultry are unable to digest all of the nutrients in the diet they consume due to the presence of NSPs and fiber in the ingredients that reduce the effectiveness of the digestive process, or they do not produce the necessary enzymes needed to degrade certain antinutritional factors in the feed. Thus, exogenous enzymes are included in poultry diets to increase the digestibility of nutrients from feed ingredients thus increasing nutrient digestion. In addition, Anadón et al. (2019) suggested that exogenous enzymes could potentially be used as alternatives to antibiotics for improving the growth performance of poultry and swine. Feed enzymes are reported to degrade NSP in by-product ingredients, thus reducing the otherwise undigested substrates and antinutritional factors resulting in the production of oligosaccharides with potential prebiotic effects in poultry (Anadón et al., 2019). As a result, wheat by-product ingredients which are relatively in-expensive could be incorporated into poultry feed without adversely affecting performance by degrading crude fiber, starch, proteins, and phytates.

A major factor influencing the use of exogenous enzymes in poultry feed is their ability to breakdown indigestible components of feed ingredients leading to improved efficiency of meat and egg production. According to Adeola and Cowieson (2011), feed enzymes are substrate-specific, targeting specific chemical bonds present in the undigestible components of feed ingredients resulting in improved digestion efficiency. Previously exogenous enzymes have been used to increase the digestibility of poorly digested diets with greater benefits from ingredients containing relatively higher amounts of fiber (Bedford, 2000).

Feed enzymes are typically produced from microbial and plant sources. However, commercial feed enzymes are produced primarily with microorganisms including Bacteria (*Bacillus subtilis*, *B. lentus*, *B. amyloliquifaciens* and *B. stearothermophils*); Fungus (*Triochoderma longibrachiatum, Asperigillus oryzae* and *Asperigillus niger*); and Yeast (*S. cerevisiae*). A common trait of all of these feed enzymes is their ability to break down plant cell wall components thus increasing the digestibility of nutrients (Bhat, 2000). The major feed enzymes commercially used in poultry production is presented in Table 2.3.

Classification of Enzymes

Enzymes are classified into six groups based on the reaction they catalyze and denoted by an EC (Enzyme Commission) number. According to Bayindirli (2010), "the first, second, and third–fourth digits of these numbers show class of the enzyme, type of the bond involved in the reaction, and specificity of the bond, respectively". Table 2.4. gives a summary of the classes of enzymes and some examples. According to Gadde et al. (2017), the possible mode of action of feed enzymes includes:

1) Digestion of nutrients not degradable by the host enzymes.
2) Elimination of the cell wall components that encapsulate nutrients, thus releasing nutrients that otherwise cannot be liberated by grinding and pelleting the cereals.
3) Inactivation of antinutritional factors particularly soluble NSPs and phytates/ phytic acids.
4) Increasing the solubility of insoluble NSPs that could increase fermentation in the ceca.
5) Supplementing the endogenous enzymes of specifically young animals which are unable to synthesize adequate endogenous enzymes for efficient digestion.

In animal nutrition, the exogenous enzymes utilized in feed formulations are classified based on the substrate they degrade and are broadly categorized into carbohydrates (starch and NSPs), proteins, lipids, and phytates degrading enzymes.

Carbohydrases break down carbohydrates in feed into oligosaccharides and monosaccharides thus increasing the amount of nutrients required for energy and growth by animals. Carbohydrases can be used to eliminate the negative effects associated with NSPs in wheat by-product ingredients thus increasing the digestibility and utilization of otherwise unavailable nutrients. As a result of greater substrate for enzyme activity, carbohydrases are usually effective in diets containing high NSPs, and as such, more beneficial in diets formulated with cereal by-products, especially wheat by-products. They can be broadly grouped into two based on their target substrate, that is starch or non-starch polysaccharides (fiber).

Starch degrading enzymes are groups of enzymes that break down starch polymers (amylose and amylopectin) in poultry feed. Starch polymers consist of glucose units linked together by α-1,4-glycosidic bonds. Amylose is predominantly a linear polymer linked mainly by α-1,4-glycosidic bonds with a few branches linked by α-1,6-glycosidic bonds (Isaksen et al., 2011). It constitutes 20 – 30% of a starch molecule and is generally hydrolyzed by endogenous amylase produced by monogastric animals. Amylopectin, in contrast, is highly branched and consists of chains of glucose linked together mainly by α-1,4-linkages and with α-1,6 bonds at the branch points. Amylopectin constitutes 70 – 80% of a starch molecule.

Most monogastric animals including poultry are able to break down these linkages. However, the degree of degradability of the α-1,4-glycosidic bonds in starch depends on the levels of resistant starch, starch granule size, starch composition, and starch encapsulation (Bedford and Partridge, 2011). In addition, the type of starch, namely rapidly degraded starch, slowly digested starch, or resistant starch affects the rate of digestibility. The hydrolysis of starch by enzymes in the small intestine of poultry and other livestock produces glucose as the final product to be absorbed directly by the intestinal epithelium.

Of the starch degrading enzymes, α-amylases are the most widely used in animal nutrition. They are included in poultry diets to hydrolyze α-1,4 glycosidic bonds of polysaccharides, leading to the production of short-chain dextrins and simpler sugars (Sindhu, et al., 2017). The benefits of exogenous amylases in poultry nutrition are well established. Bedford and Partridge (2011) reported that amylases increase starch digestibility thus allowing pigs and poultry to extract more energy from the feed for the production of meat and eggs. Supplementing broiler diets with α-amylases has resulted in increased digestibility of starch and organic matter, AME (apparent metabolizable energy), and improved feed conversion (Onderci et al., 2006; Jiang et al., 2008). Other researchers have demonstrated that supplementing corn-soy diets with exogenous amylase improved broiler performance at 7, 21, and 42 d, and increased the digestibility of starch and the AMEn (Gracia et al., 2003). Stefanello et al. (2019) conducted a study to evaluate the effects of a supplemental α-amylase on energy and nutrient utilization of broilers fed diets with variable amounts of corn. Treatment diets with or without the enzyme supplement were fed to broilers at 25 d of age. The authors reported that birds fed the diets supplemented with α-amylase had increased BW gain and improved FCR compared to those fed diets without amylase. The authors also reported an improvement in ileal digestible energy (IDE), AME, and apparent metabolizable energy corrected for nitrogen (AMEn) in diets supplemented with amylase resulting in an overall improvement in broiler performance. Additionally, supplementation of α-amylase has resulted in improvements in growth performance of young birds and subsequent growth phases (Vieira et al., 2015; Stefanello et al., 2016; 2017).

NSP degrading enzymes hydrolyze plant cell wall materials, which are indigestible by the host animals' endogenous enzymes. In feed, NSP enzymes have typically been classified based to the IUB Enzyme Nomenclature (Bairoch, 2000) and belong to the glycosyl hydrolases (EC 3.2.1.x). The classification of NSP enzymes depends on both the reaction type and substrate specificity, e.g. xylanases hydrolyze xylan present in most wheat-based feeds (Paloheimo et al., 2011). The majority of the glycosyl hydrolases enzymes are endo-acting enzymes, cutting in the middle of the polymer chain and rapidly reducing viscosity (Paloheimo et al., 2011). In poultry nutrition, NSP degrading enzymes are usually used as feed additives for young poultry fed cereal based diets. The mode of actions of NSP degrading enzymes include:

1) Hydrolysis of NSPs in the upper digestive tract decreasing digesta viscosity in the small intestine.
2) Increased nutrient digestion and absorption.
3) Metabolic responses of the intestinal tissues.
4) Modifications of quantity and composition of the intestinal microflora.

The influence of NSP degrading enzymes on intestinal microbiota occur mainly through two mechanisms: 1) removal of fermentable starch and protein through increased digestion, and 2) provision of soluble, fermentable oligosaccharides as a result of depolymerization of insoluble fiber (Choct et al.,1996; Bedford, 1996, 2000b). In feedstuffs the types and concentrations of NSP vary greatly.

Table 2.3. Type of commercial feed enzymes and target substrates

Enzyme	Target Substrate	Target Feedstuff
Phytases	Phytic acid	All plant-derived ingredients
Glucanases	Glucan	Barley, oats, and rye
Xylanases	Arabinoxylans	Wheat, rye, triticale, barley, fibrous plant materials
Galactosidases	Oligosaccharides	Soybean meal, grain legumes
Amylases	Starch	Lipids in feed ingredients
Mannanases, cellulases, hemicellulases, pectinases	Cell wall matrix (fiber components)	Plant-derived ingredients, fibrous plant materials
Lipases	Lipids	Lipids in feed ingredients
Proteases	Proteins	All plant protein sources

Source: Ravindran (2013).

Table 2.4. Classes of enzymes and some examples

EC Class	**Example**
EC1: Oxidoreductases	Peroxidase, Catalase Polyphenol oxidase, Lipoxygenase, Ascorbic acid oxidase, Glucose oxidase, Alcohol dehydrogenase
EC2: Transferases	Amylosucrase, Dextransucrase, Transglutaminase
EC3: Hydrolases	Invertase, Chlorophyllase, Amylase, Cellulose, Polygalacturonase, Lipase, Galactosidase, Thermolysin
EC4: Lyases	Pectin lyase, Phenylalanine ammonia lyase, Cysteine sulfoxide lyase, Hydroperoxide lyase
EC5: Isomerase	Glucose isomerase, Carotenoid isomerase
EC6: Ligases	Hydroxycinnamate CoA ligase

Source: Bayindirli (2010).

Therefore, dietary NSP composition of the ingredient determines the types of carbohydrases often supplemented in diets to improve digestibility, nutrient availability and absorption. Common NSP degrading enzymes used in formulating poultry diets include xylanases, β-glucanases, cellulases, hemicellulases, and ß-galactosidases.

Xylanases are enzymes belonging a group of carbohydrases, classified as glycosidases, that hydrolyze the polysaccharide xylan into xylose. They are generally used alone or in combination with other enzymes known as an enzyme cocktail in poultry diets. Xylanases are the main enzymes responsible for the degradation of arabinoxylan, hydrolyzing the 1,4-b-D-xylosidic link-age between xylose residues in the backbone increasing the digestibility of the fiber components (Mendis et al., 2016). In poultry nutrition, xylanases have been used mostly as supplements to wheat-based diets for broilers to overcome the anti-nutritional effects of NSP. The use of xylanase in poultry diets is beneficial because it can break down plant cell wall and release encapsulated nutrients resulting in the improved digestion and animal performance (Bedford, 2000). Esmaeilipour et al. (2011) reported that xylanases could be used to improve the utilization of P, by increasing cell wall permeability or liberating bound phytate in NSP-rich diets. The supplementation of exogenous xylanase to wheat-based diets has been shown to be effective in lowering the viscosity of intestinal contents and improving the digestibility of nutrients in broilers, leading to greater AME (Choct et al., 1992; Bedford and Morgan, 1996). However, the improvement in animal performance depends on the type of ingredients and source of xylanase. Nonetheless, several studies involving the supplementation of xylanase have resulted in improved animal performance. In addition, Bedford and Classen (1992) reported the capacity of xylanase to prevent the formation of viscous digesta by partially hydrolyzing NSP in the upper digestive tract, thus decreasing digesta viscosity in the small intestine. Lui and Kim

(2017) evaluated the effects of dietary xylanase supplementation in broilers fed wheat-based diets on performance and functional digestive parameters including ileal digesta viscosity, apparent ileal digestibility, intestinal morphology and microflora, digestive enzyme activities, and excreta odor content. The researchers fed diets with increasing levels of supplemental xylanase (0, 1,875, 3,750, and 5,625 XU/kg xylanase) to birds and reported that dietary xylanase supplementation increased BWG and improved FCR compared to the un-supplemented diet. The addition of xylanase also linearly decreased ileal digesta viscosity and excreta odor, and increased apparent ileal digestibility of DM, CP, gross energy, and most amino. The villus height and the ratio of villus height to crypt depth of the duodenum, jejunum, and ileum improved with increasing the level of xylanase in the diet.

β-Glucanases are commercially used in poultry nutrition to hydrolyze β-glucans. Several benefits have been demonstrated with the addition of β-glucanase enzymes to barley-based broiler diets. For instance, Chesson (1993) demonstrated that the inclusion of glucanase in poultry diets improved the efficiency of feed utilization and contributed to a better use of low-cost feed ingredients. In addition, Gohl et al (1978) observed a reduction in sticky droppings in poultry fed barley-based diets supplemented with β-glucanase. Other researchers have also suggested that the use of β-glucanase improves the digestion and absorption of starch, protein, and fat by reducing viscosity in the GIT (Annison, 1992; Bedford and Classen, 1992). Increased broiler performance and decreased intestinal viscosity in the gizzard and small intestine of birds fed barley-based diets have been associated with β-glucanase enzymes supplementation (Classen et al., 1985; Elwinger and Saterby, 1987; Marquardt et al., 1994).

Phytases belong to a subgroup of phosphomonoesterases that are capable of initiating the stepwise dephosphorylation of phytate, the most abundant inositol phosphate in nature (Greiner and Konietzny, 2011). In the poultry industry, phytase is one of the most widely researched enzymes and has been extensively used to target the substrate phytate or phytic acid. Most poultry diets are plant-based and half of the total P in these ingredients exists as phytate which is poorly utilized by poultry (Nelson et al., 1971; Waldroup et al., 2000). Therefore, exogenous phytase supplementation of poultry diets is an effective method of improving P digestibility (Wu et al., 2003; Selle and Ravindran, 2007; Adeola and Cowieson, 2011). The inclusion of phytase in poultry diets also tends to increase the digestibility of other nutrients such as calcium, sodium, and amino acids. Karimi et al. (2013) evaluated the interactions between phytase and xylanase enzymes in male broiler chicks fed phosphorus-deficient diets from 1 to 18 days of age. The authors supplemented diets deficient in available phosphorus with phytase at 0, 500, 1000, 1500, and 2000 (FTU)/kg to the. The authors reported improvements in BW, feed intake, FCR, mortality and bone ash content were dose-dependent indicating the capacity of phytase to improve growth performance and increase the available P content of diets.

In poultry, a major limitation of plant-based ingredients is the absence of endogenous enzymes for the digestion of cell wall carbohydrates (NSP and fiber) in the gastrointestinal tract. The cell wall of carbohydrates reduces the digestion of other feed components by encapsulating other nutrients and subsequently results in poor growth performance. The use of a single exogenous enzyme may not be effective due to the variability of the NSPs in wheat by-products. Therefore, enzymes are combined in the form of cocktails/multi-enzymes to improve nutrient digestion and absorption of wheat by-products in poultry diets. According to Meng et al. (2005) and Francesch and Geraert (2009) the addition of MES to broiler diets resulted in the release of metabolizable energy (ME) from otherwise unavailable sugars from NSP and P of phytate. Research involving the use of MES has been centered on xylanase, amylase, and protease (XAP) mainly due to the fact that these enzymes are able to degrade the cell walls and bound proteins associated with fibrous diets. Cowieson and Ravindran (2008b) added a mixture of XAP to broiler diets and reported that birds fed diets containing XAP had increased feed intake and BWG compared to those fed the un-supplemented diets. In a separate experiment, Cowieson and Ravindran (2008) evaluated the effects of exogenous enzymes (XAP) in corn-based diets varying in nutrient density on growth performance and digestibility of energy, minerals, and amino acids. The authors formulated two diets: a positive control diet containing adequate nutrient concentrations for broiler starters based on breeder recommendations, and a negative control diet containing approximately 0.63 MJ/kg AME and 3% amino acids less than the positive control with or without XAP enzyme product. In that study, supplementing both the positive and negative control diets with XAP improved BWG and feed efficiency compared with the un-supplemented diets. Additionally, the supplemental XAP enzyme improved performance of broilers on the negative control diet to the level of the un-supplemented positive control diet. The authors attributed the improved performance to increased nutrient digestibility through the reduction in digesta viscosity and cell wall integrity, production of fermentable disaccharides, low molecular weight polysaccharides, and improvement in protein solubility.

In addition, Flores et al. (2016) observed that using a combination of XAP enzymes together in a diet allows each enzyme to attack different poorly digestible/ insoluble NSP portions of the feed thus hydrolyzing the indigestible bonds in the plant cell wall and indigestible protein and increasing energy available for growth and/or egg production. Previous research involving the combination of phytases with carbohydrases in poultry diets in broilers fed reduced nutrient corn-SBM diets has resulted in greater enzyme efficacy resulting in improved performance (Francesh and Geraert, 2009). Other researchers have reported that the inclusion of

XAP resulted in significant increase in NSP solubility, ileal digestibility of protein, and improved nutrient utilization as compared to control diets (Olukosi et al., 2015). The increased effectiveness of XAP compared to either carbohydrase or protease alone might be due to the close fiber-protein interactions in cereals and oilseeds, thus the combination of enzymes could have an additive effect on protein, and carbohydrate hydrolysis (Olukosi et al., 2015). Because energy is the most expensive component of poultry diets, the addition of XAP enzymes to poultry diets provides producers the opportunity to reduce feed costs, by reducing the energy content of diets, without compromising on growth performance since these enzymes can liberate energy from diets.

Feed enzymes are utilized to improve the nutritional profile of feed ingredients especially cereal co-products. With the exception of phytase, exogenous enzymes hydrolyze polysaccharides to improve the energy value and digestibility of feed.

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Chapter 3 - Evaluating the effects of feeding starters crumbles on the overall performance of broilers raised for 42 days

SUMMARY

The physical form of feed affects the feed consumption and growth rate of broilers. A 42-d experiment was conducted to determine the effects of starter feed form on the growth performance of broilers. A total of 900 1-day-old Cobb 500 male broiler chicks with an average initial body weight (BW) of 38 g were randomly distributed into 36-floor pens with 25 birds per pen. Pens were randomly assigned to 1 of 4 dietary treatments within a location block to provide 9 pens per treatment. Birds were fed the dietary treatments during the starter phase from d 1 to 21 followed by a common mash diet from d 22 to 42. Dietary treatments were made up of 1) 21 d mash (M); 2) 7 d crumbles followed by 14 d mash (7C); 3) 14 d crumbles followed by 7 d mash (14C), and 4) 21 d crumbles (21C). Overall, the body weight (BW), average daily gain (ADG), and average daily feed intake (ADFI) were increased ($P < 0.001$) for the birds fed crumbles compared to those fed the mash diet. Feed conversion ratio (FCR), adjusted for mortality, was improved ($P < 0.001$) for the birds fed crumbles compared to those fed the mash diet. Birds fed 21C were heavier at 21 d and 42 d and had improved ($P < 0.001$) FCR compared to those fed the other diets. The results of the study suggest that feeding broiler chicks crumbles as opposed to a mash diet for a minimum of 7 d improved BW, ADG, ADFI, and FCR.

DESCRIPTION OF THE PROBLEM

Broiler feed intake and growth performance are significantly influenced by the physical form of the feed (Moran, 1989; Dozier et al., 2010). The particle size of the grain and feed form affect bird performance, especially during the early stages of growth. However, the effect of the particle size of diets on broiler performance is confounded by the complexity of the diet and further processing methods, including pelleting and crumbling (Sogunle et al., 2013). Even though further processing of feed into pellets increases the cost of feed, the improved performance of birds offsets the added cost (Behnke and Beyer, 2002).

Research has indicated that early chick growth has a significant impact on the final BW of broilers. Therefore, it is important to initiate faster chick growth (Behnke and Beyer, 2002) by feeding chicks more palatable and digestible feed to help optimize their growth potential and subsequent performance (Omede and Iji, 2018). Thus, the physical form of feed (mash, pellets or crumbles) is important as it affects feed intake and the subsequent growth performance of broiler chickens.

The cost of manufacturing mash feed is less expensive because it involves less processing steps and low energy inputs compared to crumbles. However, Jahan et al., (2006) suggested that mash feed is less palatable to broilers compared to crumbles, thus reducing feed intake. For this reason, broiler chicks are usually fed crumbled pellets to stimulate feed intake.

Research has shown that feeding broilers pellets or crumbles stimulates feed intake resulting in higher body weight and improved feed conversion compared to mash diets (Amerah et al., 2008; Chewning et al., 2012). The improved performance may be attributed to decreased feed wastage, reduced selective feeding, increased palatability and digestibility of feed, decreased ingredient segregation, reduction of time and energy spent during prehension, thermal modification of starch and protein, and destruction of pathogenic organisms (Behnke, 1994; 1998). In addition, Ghazi et al., (2012) suggested that pelleting tended to increase the bulk density of feed, improve feed flowability, and provide an opportunity for the addition of alternative ingredients. However, since starter chicks are unable to consume pellets due to the small size of their beaks, they are fed crumbles or small/micro pellets instead. Crumbles are pellets that have been crushed to produce a consistent form that is coarser than mash using a pair of steel rolls. Therefore, the objective of this study was to investigate the effects of feeding crumbles to broiler chicks for the first 21 d post-hatch compared to feeding them a mash diet within the same period, on their subsequent growth performance.

MATERIALS AND METHODS

The study was carried out at the Poultry Research Center of the Animal Sciences and Industry Department, Kansas State University (KSU), Manhattan, KS.

Dietary Treatments

A three-phase feeding program was used in a 42-day experiment. Diets were formulated to meet or exceed the requirements for the starter, grower, and finisher phases of the birds (Cobb 500, Cobb-Vantress Inc., Siloam Springs, AR). The starter diets were fed either in the form of mash or crumbles for 21 d. Four treatments were evaluated from 0 to 21 d of age and consisted of 1) 21 d mash (M); 2) 7 d crumbles followed by 14 d mash (7C); 3) 14 d crumbles followed by 7 d mash (14C); and 4) 21 d crumbles (21C). A common grower and finisher diet were fed as mash to all birds from d 22 to 35 and d 36 to 42, respectively.

Feed Formulation and Manufacturing

The starter, grower, and finisher diets (Table 3.1) were all manufactured at the O. H. Kruse Feed Technology Innovation Center, Kansas State University (KSU), in accordance with

Current Good Manufacturing Practices (CGMPs). The corn for the experimental diets was ground to an average particle size of 570, 650, and 720 µm using a 3-high roller mill (Model RMS 924, Sioux Falls, SD) for the starter, grower, and finisher diets respectively.

The diets were mixed in a 907 kg mixer (Model TRDB63-0152, Hayes & Stolz Ind. Mfg. Co. Fort Worth, TX). A sub-lot of the starter mash diet (M) was conditioned at 80ºC for 45 seconds in a 37 cm x 98 cm conditioner and pelleted with a pellet mill (Model CL-5, California Pellet Mill Co., Crawfordsville, IN) equipped with a 4.76 mm x 36.75 mm pellet die. The production rate was held constant at 82 kg/hr. The pellets produced were cooled with ambient air in an experimental counter-flow cooler for 10 minutes. Whole pellets were crumbled with a 17.8 cm x 20.3 cm single pair crumble roll (Model ECO ROLL 7, Eco-Roll, Colorado Mill Equipment, LLC, CO). The crumbles were fed as manufactured with no fines removed.

Particle Size and Pellet Durability Index Analysis

Particle Size Analysis. Corn samples obtained from the roller mill were analyzed using the ANSI/ASAE S319.4 standard method for particle size analysis with 0.5 g of dispersing agent and run for 10 minutes to obtain the geometric mean diameter (d_{gw}) and geometric standard deviation (S_{gw}) (Kalivoda et al., 2017).

Pellet Durability Index (PDI). PDI was determined using the Holmen pellet tester (Model NHP 100, TekPro, Norfolk, GBR). Five samples were collected at equally spaced time intervals of 10 minutes from the pellet mill. Each sample weighed approximately 500 g. Hot pellet samples were immediately cooled using ambient air in an experimental counterflow cooler for 10 minutes. Samples were then pooled together to form a composite sample and stored for PDI analysis. A 100 g sample of screened pellets was placed into the test chamber and agitated with forced air for 60 seconds and then weighed. The final weight of pellets was then expressed as a percentage of the initial whole pellets to determine the pellet durability.

Birds and Management

The Institutional Animal Care and Use Committee (IACUC) of KSU approved all experimental procedures. The study was carried out at the KSU Poultry Teaching and Research Facility, Manhattan, KS. A total of 900 one-day-old male Cobb 500 broiler chicks obtained from a commercial hatchery with an average initial BW of 38 g were randomly distributed into 36-floor pens in an environmentally controlled curtain sided house. Twenty-five birds were placed in pens measuring 2.3 x 1.5 m (L x W: 3.5 m^2) to give a stocking density of 7 birds/m^2. Each pen had new pine litter shavings at the start of the study. Pens were equipped with 6 nipple drinkers (Chore-Time Steadi-FLOW®) and a plastic tube feeder adapted to a Model C2® Plus broiler feed pan (Chore-time). The lighting program consisted of 24 hours of light throughout the experimental period. The temperature of the house was 33ºC from placement to 7 d; 29ºC from day 8 to 14 d; 27ºC from 15 to 21 d and 25ºC thereafter. Birds had ad-libitum access to feed and water throughout the study. Feeders were shaken once a day from 1 to 7 d and twice a day from 8 to 42 d.

Data Collection

The pen was the experimental unit, and data were collected by weighing birds and feeders at 7, 14, 21, and 42 d and used to calculate ADG and ADFI. Mortality was checked daily, removed, and weighed. The mortality weight was added to the pen body weight and used to calculate ADG. The FCR was calculated as the ADFI divided by the ADG.

Statistical Analysis

The experiment was arranged in a randomized complete block design (RCBD). There were four treatments (M, 7C, 14C, and 21C), and each treatment was replicated nine times. Pen location within the environmentally controlled house was used as the blocking factor. All the experimental data collected were analyzed using the GLIMMIX procedure of SAS 9.4 (SAS Institute, 1994). Mean differences were separated by Tukey's Test at ($P \leq 0.05$).

RESULTS AND DISCUSSION

Pellet Durability Index

Pellet quality as determined by the PDI test for the pellets used to create the crumbles in the study was 74%.

Growth Performance

The overall mortality was 2.44 % with no evidence of differences due to treatment effects. The effect of feed form on the growth performance of broilers during the 42 d experimental period is presented in Tables 3.2 and 3.3. Feed form affected BW, ADG, ADFI, and FCR of broilers throughout the study. The results of d 0 to 7 and 0 to 14 were similar. During these periods, birds fed crumbles had increased ($P < 0.001$) BW, ADG, ADFI, and improved ($P < 0.001$) FCR compared to those fed the mash diet. However, no evidence of a difference ($P > 0.05$) in FCR was observed in birds fed 7C and M at 14 d.

By d 21 birds fed crumbles had increased ($P < 0.001$) BW compared to those fed the mash diet. However, the birds fed 21C diet had the highest ($P < 0.001$) BW compared to those fed the other treatment diets. Additionally, ADG and ADFI were increased ($P < 0.001$) whereas FCR was improved ($P < 0.001$) for birds fed crumbles compared to those fed the mash at 21 d.

Birds fed crumbles in the starter phase had an increased (*P* < 0.001) overall BW compared to those fed the mash diet. Again, the overall ADG and ADFI were increased (*P* < 0.001) for birds fed crumbles compared to those fed the mash diet. Additionally, the FCR was improved for birds fed crumbles 7C (1.51), 14C (1.51), 21C (1.49) compared to those fed the mash diet, M (1.53) for 7C, 14C, 21C at 42 d. Moreover, the greatest improvement in FCR at 42 d (*P* < 0.001) was found in birds fed 21C.

The results of the study indicated that feeding crumbled diets increased feed intake that resulted in improved growth performance of broilers. During the first 7 d, there was a 6.5% increase in feed intake resulting in a 10.4% improvement in BW for broilers fed crumbles compared to those fed the mash diet. Again, a 6.6% improvement in feed efficiency was observed in birds fed crumbles compared to those fed the mash diet at 7 d post-hatch. The observed improvement in feed intake, BW, and feed efficiency could be attributed to decreased feed wastage due to less feed falling from the beak into the water or onto the floor. Other authors have reported similar results with feeding pellets or crumbles vs mash diets to birds and have attributed the improved performance to decreased feed wastage and ingredient segregation, reduction in selective feeding, decreased time and energy spent during prehension and, increased palatability and digestibility of feed due to pelleting (Moran, 1989; Behnke, 1998; Ghazi et al., 2012; Chewning et al., 2012).

In the current study, feed intake and BW at 21 d post-hatch increased for birds fed crumbles compared to those fed the mash diet. Birds fed 21C had an 8% increase in feed intake which lead to a 10.3% increase in BW compared to those fed the mash diet. The improvement in BW could be attributed to pelleting which increase feed intake, and subsequently stimulated early chick growth resulting in higher growth performance. This agrees with other researchers who observed similar findings. For instance, Amornthewaphat et al. (2005) reported that birds fed crumbles had significantly greater BW compared to those fed mash diet at 21 d of age. The authors attributed the improvement in bird performance to higher feed and nutrient intake due to increased nutrient density in pellets, improved starch digestibility, and reduced feed wastage. However, Moran (1989) and McKinney and Teeter (2004) suggested that improved growth performance of birds fed pellets might be due to less time and energy spent during prehension. Feed form had significant effect on the overall BW, ADG, ADFI, and FCR. Broilers fed crumbles had heavier BW compared to those fed mash diet that translated to between (4.9% to 5.6%) over the experimental period. The results were consistent with the findings of Calet (1965); Choi et al. (1986) and Nir et al. (1994a; 1994b; 1995) who reported that feeding pelleted diets improved broiler growth rate and bird performance compared to when mash diets were fed. Dozier et al. (2010) also observed a higher growth rate in birds fed high-quality pellets compared to those fed mash diets from 15 to 42 d of age. Furthermore, Kim and Chung (1994); Van Biljon, (2005); Jahan et al. (2006) and Zakeri et al. (2013) reported that broiler chickens fed crumble pellet diets had significantly heavier BW at 42 d when compared with birds fed all-mash.

Research has shown that feed consumption is the major driver of body weight gain of broilers. Throughout the study period broilers fed crumbles had increased feed intake compared to those fed the mash diet accounting for the higher BW observed. Hu et al. (2012) investigated the effect of feeding crumbles and pellet quality on broiler performance and gizzard weight and demonstrated that birds fed crumbles had a higher feed intake which led to heavier BW gain compared to those fed the mash diet. Additionally, Zang et al. (2009) reported a 7 to 10% higher feed intake in birds fed crumbles compared to those fed mash diets and attributed the higher feed intake to reduction in selective feeding and increased nutrient digestibility of the crumble diets.

In a more recent study, Omede and Iji (2018) investigated the response of broiler chickens to processed soy protein product when offered at different inclusion levels in a mash or crumbled prestarter diets and observed that birds fed crumbles had higher feed intake which resulted in higher BW gain compared to those fed the mash diet at 24 d post-hatch. The increased feed intake was attributed to the larger sizes of crumbles compared to mash diet which reduced selective feeding. This is consistent with other researchers who suggested that birds prefer larger feed particles and tend to select feed based on the size of their oral cavity (Portella et al., 1988).

It is suggested that feeding crumbles to broilers improves feed efficiency by reducing feed wastage due to less feed particles falling from the beak into the water or onto the floor (Jensen, 2000). In the current study, there was significant improvement in FCR for birds fed crumbles compared to those fed the mash diet. Feeding broilers crumbles for 21 d resulted in 2.68% improvement in FCR (1.49 crumbles vs 1.53 mash) compared to feeding mash diet at 42 d. Other researchers have reported improvement in FCR when crumbles vs mash diets were fed to broilers: 8.4% (1.85 vs 2.02) Chewing et al. (2012) and 8.9% (1.52 and 1.67) (Amerah et al., 2007).

The results from the study suggested that feeding crumbles to starter birds increased feed intake which stimulated early chick growth and subsequently improved overall growth performance and feed efficiency of birds compared to feeding a mash diet.

CONCLUSION AND APPLICATIONS

1. Feeding crumbles to broilers improved feed intake which stimulated early chick growth and resulted in improved BW and feed conversion compared to a mash diet.
2. Birds fed the 21C had the highest BW at d 21, but the difference disappeared at 42 d of age due to compensatory growth only in the crumbled diets.

3. Feeding crumbles up to 21 d resulted in the best FCR throughout the experimental period.
4. The results of the experiment suggest that feeding crumbles improved broiler performance as compared to a mash diet.

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Table 3.1. Composition of diets by phase fed to Cobb 500 broilers from 1 d to 42 d[1].

Ingredient (%)	**Starter**	**Grower**	**Finisher**
Corn	54.63	59.25	60.23
Soybean Bean Meal, 48% CP	33.00	28.00	23.00
DDGS	2.00	2.00	6.00
Choice White Grease	5.80	6.40	6.90
L-Threonine	0.30	0.30	0.20
Monocalcium P 21%	1.82	1.70	1.36
Limestone	0.75	0.74	0.78
Salt	0.40	0.35	0.25
L-Lysine HCl	0.18	0.19	0.27
DL-Methionine	0.42	0.37	0.31
Vitamin Mineral Premix[2]	0.50	0.50	0.50
Choline chloride, 60%	0.20	0.20	0.20
Calculated Nutrient Composition			
ME, (MJ/kg)	12.62	12.97	13.12
Crude Protein, %	21.24	19.05	17.80
Crude Fat, %	8.34	9.04	9.66
Ca, %	0.89	0.84	0.76
P (Total), %	0.80	0.75	0.68
Avail. P, %	0.45	0.42	0.38
Avail. Lysine, %	1.18	1.07	0.87
Avail. Methionine, %	0.55	0.50	0.45
Avail. Methionine + Cysteine, %	0.94	0.89	0.82
Analyzed Nutrient Composition			
Crude Protein, %	23.20	22.40	20.20
Crude Fat, %	10.10	9.40	11.90
Ca, %	0.94	0.87	0.80
P (Total), %	1.01	0.59	0.75

[1] Common diet formulated for all birds with only starter diet differing in feed form.

[2] The vitamin mineral premix supplied the following per kg of complete feed: Manganese 125 mg; Zinc 150 mg; Iron, Copper, Iodine 600 ppm; Selenium 60 ppm; Vitamin A 635,600 IU; Vitamin D3 227,000 IU; Vitamin E 1,362 IU; Vitamin B12 0.9 mg; Menadione 68 mg; Riboflavin 545 mg; Thiamine 91 mg; D-Pantothenic Acid 545 mg; Niacin 2,270 mg; Vitamin B6 114 mg; Folic Acid 57 mg; Choline 31,780 mg; Biotin 3 mg.

Table 3.2. Effects of feed form on body weight (BW), of broilers reared to market age (42 d)[1]

Growth Phase	**0 – 7**	**0 – 14**	**0 – 21**	**0 – 42**
Diet	**BW (g)**	**BW (g)**	**BW (g)**	**BW (g)**
Mash	138^{b}	377^{b}	903^{c}	2924^{b}
7C	154^{a}	416^{a}	975^{b}	3114^{a}
14 C	156^{a}	428^{a}	968^{b}	3131^{a}
21 C	155^{a}	430^{a}	1007^{a}	3141^{a}
SEM[2]	2.18	5.30	9.00	22.30
P Value	< 0.001	< 0.001	< 0.001	< 0.001

[1] A total of 900 1-day-old male Cobb 500 broilers, 25 chicks per pen, 4 treatments, and 9 replicates per treatment were used.

[2] SEM: Standard error of means.

$^{a\text{-}c}$ Means within a column having different superscripts are significantly different ($P \leq 0.05$).

Table 3.3. Effects of feed form on average daily gain (ADG), average daily feed intake (ADFI), and feed conversion ratio (FCR) of broilers reared to market age (42 d)[1].

Growth Phase	**0 – 7**			**0 – 14**			**0 – 21**			**0 – 42**		
Diet	**ADFI (g)**	**ADG (g)**	**FCR (g/g)**	**ADFI (g)**	**ADG (g)**	**FCR (g/g)**	**ADFI (g)**	**ADG (g)**	**FCR (g/g)**	**ADFI (g)**	**ADG (g)**	**FCR (g/g)**
Mash	17.3^{b}	14.3^{b}	1.21^{a}	32.2^{b}	26.4^{b}	1.22^{a}	50.6^{b}	40.4^{b}	1.25^{a}	106^{b}	69^{b}	1.53^{a}
7C	18.6^{a}	16.5^{a}	1.13^{b}	34.6^{a}	29.6^{a}	1.18^{ba}	53.4^{a}	43.8^{a}	1.22^{b}	109^{a}	72^{a}	1.51^{b}
14 C	18.5^{a}	16.7^{a}	1.11^{b}	35.4^{a}	30.5^{a}	1.15^{b}	54.1^{a}	44.0^{a}	1.23^{b}	111^{a}	73^{a}	1.51^{b}
21 C	18.5^{a}	16.5^{a}	1.12^{b}	34.5^{a}	30.2^{a}	1.13^{b}	55.0^{a}	45.3^{a}	1.21^{b}	109^{a}	73^{a}	1.49^{c}
SEM[2]	0.29	0.29	0.011	0.50	0.38	0.011	0.70	0.47	0.012	0.84	0.74	0.010
P Value	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001

[1] A total of 900 1-day-old male Cobb 500 broilers, 25 chicks per pen, 4 treatments, and 9 replicates per treatment were used.

[2] SEM: Standard error of means.

[a-c] Means within a column having different superscripts are significantly different ($P \leq 0.05$).

Chapter 4 - Effects of Pellet Diameter and Crumble Size on the Growth Performance and Relative Gizzard Weight of Broilers

SUMMARY

An experiment was conducted to compare the growth performance, FCR, and relative gizzard weight (RGW) of broilers fed a mash diet, micro-pellets, or coarse and fine crumbles with or without the fines removed. A total of 300 male broiler chicks were randomly distributed into 60 battery cages with 5 broilers per cage. Cages were randomly assigned to 1 of 6 dietary treatments to provide 10 replicates per treatment. Treatments comprised of 1) mash (M); 2) micro-pellets (MP); 3) coarse-crumbles with fines (CCF); 4) coarse-crumbles with fines removed (CCNF); 5) fine-crumbles with fines (FCF); and 6) fine-crumbles with fines removed (FCNF). BW and feed intake were measured and used to calculate ADG, ADFI, and FCR at 7, 14, and 21 d. Overall BW, ADG, and ADFI were increased ($P<0.001$), FCR improved ($P<0.001$), and RGW decreased ($P<0.001$) for broilers fed MP or crumbles compared to the mash diet. BW of broilers fed MP or crumbles was greater ($P<0.001$) than those fed the mash. Broilers fed the CNF had the best FCR while birds fed the mash had poorest FCR. Broilers fed M had the highest ($P<0.001$) RGW while those fed CN had the lowest. The results of this study indicated that feeding MP or crumbles to broiler chicks improves growth performance and feed efficiency but reduces RGW. Removing fines resulted in further improvement in BW for broilers fed crumbles. Therefore, feeding good quality crumbles will yield similar growth performance in chicks as micro-pellets and does not require a pellet die change.

DESCRIPTION OF THE PROBLEM

Broiler starter diets are typically fed as mash or crumbles. Feeding crumbled pellets or micro-pellets (less than 3.0 mm) during the starter phase is a common practice in the US and other parts of the world. Research has shown that feeding broilers micro-pellets could be beneficial by increasing early growth rate and subsequent performance (Cerrate et al., 2008). Modern broilers have been genetically selected for a faster growth rate; therefore, they reach market weight in fewer days. Therefore, feed intake during the first 7 days after hatching is vital to ensure that chicks receive the nutrients required to support early chick growth.

Broiler chicks need both palatable and digestible diets in order to achieve their full growth potential especially in the early stages of the production cycle (Willemsen et al., 2008). Pellets are more palatable and stimulate feed intake and improve growth performance when fed to broiler chicks during the first few weeks of their growth cycle. However, since broiler chicks cannot consume whole pellets, the pellets are broken into crumbles. Another option is to feed micro-pellets, but this requires a die change on the pellet mill. Michard and Rouxel (2015) suggested feeding pellets of particle sizes of between 1.5 mm and 2.0 mm to broiler chicks. Rubio et al. (2017) reported that feeding chicks crumbles or 3.3 mm micro-pellets during the starter period (1 to 14 d) improved growth performance and resulted in an additional 30 g of breast meat as compared to those fed the mash diet for 35 d. The study also suggested that early chick growth has an impact on subsequent growth performance of broilers.

Although feeding crumbles improves broiler performance as compared to mash diets, crumble quality can also impact feed intake. Crumble quality is often a subjective measurement based on the size of the crumbles and presence of fine particles in the crumbles. While the presence of fines in the diet can decrease feed intake, very few studies have been conducted to quantify the effect of crumble quality on chick performance. In addition to reduced feed intake, poor quality crumbles may increase the amount of dust in the broiler house, which could create respiratory challenges and decrease growth performance.

Crumble quality can be improved by adjusting the roll gap width of the crumbler and using sharp rolls to reduce the number of fines (Stark et al., 2018). Alternatively, crumbles could be sifted mechanically with screens to remove fines and improve crumble quality. However, there are limited studies on the effect of crumble size, quality, and pellet size on post-hatch performance of broiler chickens.

The gizzard is an important organ in poultry responsible for the grinding of feed materials. Research has indicated that lack of structural components in poultry diets could result in non-functional gizzard thus affecting intestinal health (Mateos et al., 2012; Svihus, 2014). The volume of the gizzard is reported to increase in response to structural components including whole or coarsely ground cereals (Amerah et al., 2007). Aguzey and Gao (2018) reported that feeding broilers mash diets leads to a positive effect on gizzard development by increasing the relative weight compared to feeding pelleted/crumbled diets. The reduction of gizzard weight might be due to the lack of mechanical stimulation and reduced transit time in the gizzard by the pelleted/ crumbled feed (Mateos et al., 2012). Therefore, the objective of the study was to compare the growth performance, feed conversion ratio, and relative gizzard weight of broiler chicks fed diets in the form of a mash, micro-pellets, or coarse and fine crumbles either with or without the fines removed.

MATERIALS AND METHODS

The current study was carried out at the Poultry Research Center of the Animal Sciences and Industry Department, Kansas State University (KSU), Manhattan, KS.

Dietary Treatments

A common starter diet was produced and subjected to six dietary treatments with each treatment having 10 replicates. Treatments comprised of mash (M); micro-pellets (MP); coarse-crumbles with fines (CCF); coarse-crumbles with fines removed (CCNF); fine-crumbles with fines (FCF); and fine-crumbles with fines removed (FCNF).

Feed Formulation and Manufacturing

A common corn-SBM starter diet was formulated to meet or exceed the nutritional requirements of broiler chicks based on Cobb 500 standards (Table 8). The feed was manufactured at the O. H. Kruse Feed Technology Innovation Center in accordance with Current Good Manufacturing Practices (CGMPs). The corn for the experimental diets was ground to an average particle size of 530 microns using a 3 high roller mill (Model RMS 924, Sioux Falls, SD). The starter diet was mixed in a 907 kg counterpoise mixer (Model TRDB63-0152, Hayes & Stolz Ind. Mfg. Co., Fort Worth, TX) to produce the mash feed. All the fat was added to the feed in the mixer. Feed samples were collected from the complete feed during packaging and analyzed for crude protein, crude fat, and crude fiber.

A portion of the mash diet was conditioned at 82ºC and pelleted with a 1-Ton 30-HP Pellet Mill (Model 1012-2, California Pellet Mill Co., Crawfordsville, IN) equipped with a 2.38 mm x 25.4 mm or 4.76 mm x 41.28 mm pellet die to produce the MP and whole pellets used to manufacture crumble treatments, respectively. The production rate was held constant at 544 kg/hr., and the pellets were cooled to a temperature of 24ºC with ambient air using a counterflow cooler. Whole pellets produced using the 4.76 mm x 41.28 mm pellet die were crumbled with a 17.8 cm x 20.3 cm single roll pair crumble roll (Model ECO ROLL 7, Eco-Roll, Colorado Mill Equipment, LLC., CO). The crumble roll gap was adjusted to obtain two crumble sizes (2.25 mm roll gap for coarse and 1.25 mm roll gap for fine). A portion of the crumbled diet was sifted to remove the fines. The fines were removed with a SWECO Vibro-Energy Separator (Model LS18S3P3, SWECO, M-LLC, Florence, KY) equipped with either a 1.0 mm or 0.54 mm screen to obtain the coarse and fine crumbles without fines, respectively.

Particle Size Analysis. Samples of the ground corn obtained from the roller mill and samples of the mash and crumbles were obtained from the feeders and divided using a riffle divider to obtain a representative sample. Approximately 100 (± 5 g) samples were weighed and then analyzed using the ANSI/ASAE S319.4 standard method for particle size analysis with 0.5 g dispersing agent and run for 10 minutes to obtain the d_{gw} and S_{gw} (Kalivoda et al., 2017).

Pellet Durability Index (PDI). Pellet quality as determined by PDI was conducted using the New Holmen pellet tester (Model NHP 100 Portable Pellet Durability Tester, TekPro Ltd., Willow Park, North Walsham, Norfolk, UK). A 100 g sample was screened to remove the fines with either a 4.0 mm or 2.0 mm screen for the 4.76 mm and 2.38 mm pellets, respectively, and then placed into the perforated test chamber and agitated with forced air for 60 seconds. The pellets remaining in the test chamber were weighed. The final weights of pellets were then expressed as a percentage of the initial sample weight to determine the pellet quality. PDI was calculated as follows:

$$\text{PDI} = \frac{\text{Initial Pellet Weight} - \text{Final Pellet Weight}}{\text{Initial Pellet Weight}} \times 100\%$$

Bird Husbandry

All experimental procedures were conducted in accordance with the Institutional Animal Care and Use Committee (IACUC) guidelines of Kansas State University, Manhattan, KS. A total of 300 one-day-old male broiler chicks (Cobb 500, Cobb-Vantress Inc., Siloam Springs, AR) obtained from a commercial hatchery were used for the study. Broilers were individually weighed and randomly allocated according to BW to 60 Petersime battery brooder units (Model Petersime Incubator Co. Gettysburg, OH) in an environmentally controlled house with 5 chicks per cage. Each cage had a raised wire floor, trough feeder, and water. Broilers had *ad libitum* access to feed and water throughout the experimental period. The temperature of the brooders at the time the chicks were placed in them was 32ºC. This was gradually reduced to 28ºC from 1 to 7 d, then 26ºC from 8 to 14 d, and maintained at ambient temperature (25ºC) till the end of the study. Lighting was provided 24 h a day throughout the experimental period.

Data Collection

Initial cage BW was collected at placement of chicks into the brooders on d 1. Feed intake and BW were collected on 7, 14, and 21 d of age. ADG and ADFI were calculated for each cage. Mortality was checked twice daily, and broilers that died were removed and weighed and used to correct for FCR.

Gizzard Weight Measurements

On d 21, one bird with an approximate average cage BW (10 broilers per treatment) was selected, weighed, and killed by cervical dislocation. The gizzard was excised, and adhering fat trimmed. The contents were removed and then washed with clean water to remove all the digesta. The gizzard was dried using paper towels and then weighed. The gizzard weight was expressed as a percentage of the BW to determine the relative gizzard weight (RGW).

Statistical Analysis

All the data obtained during the experiment were analyzed using the GLIMMIX procedure of SAS version 9.4 (SAS Institute, 1994). The statistical model considered the effects of feed form on growth parameters and relative gizzard weight using the cage as the experimental unit. The data were analyzed as a CRD through analysis of variance (ANOVA). Means were partitioned using Tukey's test with statistical significance set at $P \leq 0.05$.

RESULTS AND DISCUSSION

Particle Size and Pellet Durability Index

The mean particle size (geometric diameter average, d_{gw}) and the geometric standard deviation (S_{gw}) of the ground corn, mash feed, and the crumbles are presented in Table 9. The particle size distribution of the mash diet and the crumbles are shown in Table 10. Pellet quality as determined by PDI for the 2.38 mm and 4.76 mm diameter pellets were 89% and 80%, respectively. The percentage fines in the micro-pellets, coarse-crumbles, and fine-crumbles were 6.50%, 8.20% and 9.03%, respectively.

Growth Performance

Mortality of the birds for the experimental period was 1.6% and was not related to any of the treatment diets. The effect of feed form on BW, ADG, ADFI, and FCR from 0 to 21 d is presented in Table 11. The RGW of broilers fed the different feed form is shown in Table 12. The BW of broilers was influenced by the physical form of the feed throughout the experimental. Throughout the study, broilers fed MP or crumbles had higher ($P < 0.001$) BW, ADG, ADFI, and improved ($P < 0.001$) FCR compared to those fed the mash diet. On d 7, the response of BW of broilers was significantly impacted by pelleting compared to the mash diet. Broilers fed crumbles had increased ($P < 0.001$) BW compared to those fed the mash diet with broilers fed MP having an intermediate BW (189 g). Additionally, the ADG was increased ($P < 0.001$) for broilers fed MP or crumbles compared to those fed the mash diet at 7 d. Similar observations were made at 7 d for the ADFI, with broilers fed the MP or crumbles having increased ($P < 0.001$) ADFI compared to those fed the mash diet. Furthermore, the FCR was improved ($P <$

0.001) for broilers fed MP or crumbles compared to those fed the mash at 7 d. However, feeding broilers MP resulted in similar ($P > 0.05$) FCR compared to feeding crumbles.

As expected, BW was increased ($P < 0.001$) for broilers fed MP or crumbles compared to birds fed the mash diet at 14 d. Similarly, ADG and ADFI within the same period increased ($P < 0.001$) for broilers fed MP or crumbles compared to those fed the mash diet. In addition, the FCR at 14 d improved ($P < 0.001$) for birds fed the MP or crumbles compared to those fed the mash diet. Moreover, no difference ($P > 0.05$) in FCR was observed between the broilers fed the MP and those fed crumbles.

Overall the BW, ADG, ADFI, and FCR were affected by the physical form of the feed presented to the broilers at 21 d. Feeding MP increased ($P < 0.001$) the BW of broilers by 15% compared to feeding the mash diet. Similarly, the BW increased ($P < 0.001$) by 10.4 to 14.6% for broilers fed the crumbles compared to those fed the mash diet. Removing the fines from the crumbles resulted in a further increase in BW of between 1.4 to 4.7% for the broilers fed the crumbles. Furthermore, ADG and ADFI were increased ($P < 0.001$) at d 21 for broilers fed MP or crumbles compared to those fed the mash diet. The FCR improved ($P < 0.001$) for broilers fed MP or crumbles compared to those fed the mash diet at 21 d. Compared to the mash diet, feeding MP (1.29 vs 1.37) improved FCR by 8.4% whereas feeding crumbles (1.24 vs 1.37) improved FCR by 8.5%. However, the best improvement in FCR of 13.8% was recorded by broilers fed CNF (1.24 vs 1.37) compared to the mash diet. The results of the study suggested that feeding MP or crumbles resulted in increased BW, ADG, and ADFI, and improved FCR compared to feeding a mash diet. The study also indicated that feeding a good quality crumbled diet will yield similar growth performance in broilers as a micro pellet and does not require a pellet die change.

Relative Gizzard Weight

Table 12 shows the effect of feed physical form on the RGW of the broilers. From the study, the RGW was impacted ($P < 0.001$) by the physical form of the feed presented to the broilers at 21 d. The RGW was increased ($P < 0.001$) for broilers fed the mash diet compared to those fed MP or crumbles. Interestingly, broilers fed fine-crumbles (with or without fines removed) had increased ($P < 0.001$) RGW compared to those fed MP or coarse-crumbles (with or without fines removed). The results of the study indicated that feeding a mash diet stimulated gizzard activity thus resulting in heavier gizzards compared to micro-pellets or crumbles.

Studies outlining the benefits of feeding pellets or crumbles on broiler performance are well documented (Moran, 1989; Behnke, 1994; McKinney and Teeter, 2004; Cerrate et al., 2008; Chewning et al., 2012; Xu et al., 2015). Feeding broiler chickens pellets or crumbles improves broiler performance by increasing feed intake, which results in improved growth performance and FCR (Abdollahi et al., 2013). The improvement in performance has been credited to decreased feed wastage, reduced selective feeding, decreased ingredient segregation, increased palatability, and thermal modification of starch and protein (Behnke, 1994).

The growth performance results obtained in the current study showed that feeding MP or crumbles increased BW, ADFI, and improved FCR of broilers compared to the mash diet. On d 7, feeding MP or crumbles improved growth rate, feed intake, and FCR of broilers compared to feeding the mash diet. However, pellet or crumble size had little or no impact on BW, feed intake, and FCR of broilers fed the pelleted diets during this growth period. The improvement in BW observed in the broilers fed either the MP or crumbles was driven primarily by feed intake. Although feeding MP (2.38 mm) slightly reduced the BW and increased the FCR of broilers, feed intake was similar to those fed the crumble diets (1.25 or 2.25 mm). In addition, feeding the different crumbled diets yielded similar growth performance. Therefore, both crumble size and quality had little influence on the performance of broilers fed the pelleted diets at 7 d. In accordance with the present study, Engberg et al. (2002) and Svihus et al. (2004) indicated that feed intake drives BW gain and is considered as the main reason for pelleting broiler diets. Research has shown that the size of the pelleted diets selected by broilers depends on the variations in their oral cavity (Moran, 1989) with small chicks preferring sizes of between 1.5 mm and 2.0 mm (Michard and Rouxel, 2015). This agreed with the results obtained from the present study, where broilers fed crumbles of sizes between 1.25 and 2.25 mm had slightly increased feed intake, which resulted in higher BW compared to those fed MP at 7 d. This suggested that the crumble size offered might be suitable for the oral cavity of broilers at this age compared to the MP.

On 14 d, BW was increased for broilers fed MP or crumbles compared to those fed the mash diet. This was similar to those obtained by Hu et al. (2012), who reported heavier BW for broilers fed crumbles compared to those fed the mash diet at 14 d. In addition, Amerah et al. (2007) reported that pelleting increased nutrient intake and decreased energy expenditure during prehension resulting in improved broiler performance. In accordance with the results obtained by Hu et al. (2012), feed intake was significantly increased for broilers fed MP or crumbles compared to those fed the mash diet at 14 d. The ADFI, ADG, and FCR were not influenced by pellet or crumble size in broilers fed the pellets or crumbles. However, crumble quality had a remarkable impact on BW only in broilers fed the fine crumbled diets. Broilers fed the FCF diet had the lowest BW compared to the other crumbled diets. In a more recent study, Lemons et al. (2019) evaluated the interaction of feed form and phase of feeding on performance of Ross 708 male broilers throughout a 46 d grow-out period. The authors demonstrated that broilers presented with crumbles (1785μm) had increased feed intake, improved FCR and a 9 g improvement in BWG at 14 d compared to broilers fed ground crumbles (988 μm). The improvement in performance (BW and FCR) was attributed to increased feed intake of the crumble-fed broilers due to better feed integrity compared to the ground crumbles, which is in agreement with results of the current study. This is contrary to the results reported by Cerrate et al. (2008) who observed no difference in BW at 14 d for broilers fed crumbles produced with a 3.17- or 1.59-mm die, even though the percentage of fines in the diets differed. In addition, feed form had a significant influence on FCR, with broilers fed MP or crumbles having improved performance compared to those fed the mash diet. Similar BW, feed intake, and FCR between broilers fed MP and crumbles indicated that the MP might be suitable for the oral cavity at 14 d of age. The data from the current study demonstrated that feeding MP yielded similar performance as crumbles at 14 d of age.

The overall broiler performance at 21 d showed increased BW, ADG, ADFI, and improved FCR for broilers fed MP or crumbles compared to those fed the mash diet. This is consistent with the findings of other researchers (Jones et al., 1995; Amornthewaphat et al., 2005; Amerah et al., 2007; Hu et al., 2012; Rubio et al., 2017). From the study, feeding crumbles or MP resulted in 10.3% (994 g vs 891 g) to 15.1% (1049 g vs 891 g) increase in BW as compared to the mash diet. In addition, the feed intake was increased between 6 to 10% for broilers fed crumbles or MP compared to broilers fed the mash diet. This agreed with the results obtained by Amornthewaphat et al. (2005) who reported that broilers fed crumbles exhibited significantly higher feed intake compared to those fed the mash diet at 21 d of age. The improvement in feed intake of broilers fed micro-pellets or crumbles might be attributed to decreased selective feeding behavior of broilers due to better feed integrity compared to mash feed (Abdollahi et al., 2013; Xu et al., 2015). In most broiler production systems, an increase in feed intake is the major goal since it results in higher BW gain. The data from the current study is consistent with these authors. For instance, Abdollahi et al. (2011) reported a 14% increase in feed intake in broilers fed pelleted diet during the starter phase (1 to 21 d) compared to broilers fed the mash diet. Furthermore, Jensen (2000) suggested that pelleting increases feed intake due to the reduction in feed wastage due to fewer particles falling from the beak onto the floor or into the water.

It is well established that feeding pellets or crumbles to broilers results in improved feed efficiency compared to a mash diet. Nir et al. (1994b) reported improved feed efficiency in broilers fed pelleted diets compared to a mash diet and attributed the performance partly to a reduction in feed energy used for maintenance. Other researchers have attributed the improved feed efficiency to less energy spent during feeding (McKinney and Teeter, 2004; Skinner-Noble et al., 2005). In agreement with these authors, the FCR of the current study was improved by feeding either MP or crumbles to broilers compared to the mash diet.

Crumble quality has a significant impact on broiler growth performance. From the current study, broilers fed crumbles with fines removed outperformed those fed crumbles containing fines. In terms of BW, crumble quality affected broiler performance for the different crumble sizes. Removing fines in both coarse and fine crumbles increased BW by 3% in broilers fed the crumbled diets with similar feed intake. This agreed with Plavnik et al. (2002) who reported that increasing the level of fines or grinding pellets negatively affected broiler performance. The results indicated that high levels of fines in the crumbles reduced feed intake, which resulted in lower BW and poorer FCR. In the current study feeding CCNF improved FCR by 4% compared to that of the CCF. However, Corzo et al. (2011) observed no difference in performance when diets containing 32% and 64% pellets were fed to broilers. From the current study, feeding micro-pellets resulted in similar performance (BW) as feeding crumbles to 21 d of age.

Poultry do not have teeth, therefore the gizzard is used to break down the feed materials in the GIT of the broiler. Gizzard is development is therefore essential for the effective digestion in broilers. Aguzey and Gao, 2018 reported that feeding broilers mash diets had a positive effect on gizzard development and resulted in a larger relative gizzard weight. From the current study, the RGW was larger in broilers fed the mash diet as compared to those fed MP or crumbles at 21 d of age. This agreed with Dahlke et al. (2003) who reported that a mash diet stimulated the gizzard, which resulted in a larger gizzard as well as increased the retention time.

Svihus et al (2004) reported a decrease in RGW reduction when broiler was switched from mash to pelleted diets. From the current study, feeding MP or crumbles resulted in decreased RGW compared to the mash diet. Abdollahi et al., 2013 suggested that the pelleting process reduced the particle size of the feed, which resulted in a smaller gizzard. Choi et al (1986) and Nir et al (1994a, b) reported that feeding crumbles or pellets resulted in a reduction of the relative weight (g/kg of BW) of the gizzard compared with feeding mash diets. These further reduce the retention time and increase the passage rate of digesta in the gizzard, resulting in less mechanical stimulation subsequently reducing the gizzard size (Mateos et al., 2012). Engberg et al. (2002) suggested that less stimulation of the gizzard occurred as a result of low feed intake in broilers fed pelleted diets, which could be the reason for the relatively smaller gizzard weight compared to mash-fed broilers. This is consistent with the results of the current study where the RGW was increased for broilers fed mash diets compared to those fed MP or crumbles. However, the heavier gizzard weights observed in broilers fed fine-crumbles compared to the micro-pellets or coarse-crumbles could not be immediately explained. Perhaps the presence of smaller particles resulted in higher feed volume in the gizzard stimulating the activity of the gizzard, which subsequently increased the size. The results of the current study indicated that feeding a mash diet encourages gizzard development as opposed to feeding a crumbled diet

CONCLUSIONS AND APPLICATIONS

1. Feeding micro-pellets or crumbles increased feed intake, thus improving growth performance and feed conversion ratio of broilers compared to the mash diet.
2. Feeding micro-pellets yielded similar growth performance in broiler chicks as good crumbles and thus could be utilized as a starter diet.
3. Growth performance was not affected by the crumble size, but crumble quality resulted in further improvement in growth performance of broilers fed the crumble diets.
4. Feeding a mash diet stimulated gizzard activity which subsequently resulted in heavier relative gizzard weight compared to micro-pellets or crumbles.

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Table 4.1. Composition of diet fed to Cobb 500 broilers from 1 to 21 d[1]

Ingredient, %	**Starter**
Corn	60.14
SBM 46.5 %	32.50
Soy Oil	3.00
L-lysine HCl	0.16
DL-Methionine	0.28
L- Threonine	0.08
Monocalcium P 21%	1.80
Limestone	1.23
Salt	0.23
Vitamin Mineral Premix[1]	0.25
Sodium Bicarbonate	0.23
Choline Chloride 60%	0.10
Total	100.00
Calculated Nutrient Composition	
Dry Matter, %	86.99
ME, (kcal/kg)	3097
TMEn, (kcal/kg)	3171
Crude Protein, %	21.13
Crude Fat, %	3.32
Avail. Lysine, %	1.13
Avail. Methionine, %	0.57
Avail. Threonine, %	0.74
Calcium, %	0.88
Phosphorus, %	0.76
Avail. Phosphorus, %	0.45
Analyzed Nutrient Composition, %	
Dry Matter	100.00
Crude Protein	24.00
Crude Fat	5.60
Crude Fiber	3.60

[1] Common corn-SBM-based diet formulated for broiler starters and contained three different feed forms (mash, crumbles and micro pellets).

[2] The vitamin mineral premix supplied the following per kg of complete feed: Manganese 125 mg; Zinc 150 mg; Iron, Copper, Iodine 600 ppm; Selenium 60 ppm; Vitamin A 635,600 IU; Vitamin D3 227,000 IU; Vitamin E 1,362 IU; Vitamin B12 0.9 mg; Menadione 68 mg; Riboflavin 545 mg; Thiamine 91 mg; D-Pantothenic Acid 545 mg; Niacin 2,270 mg; Vitamin B6 114 mg; Folic Acid 57 mg; Choline 31,780 mg; Biotin 3 mg.

Table 4.2. Particle size of ground corn and the crumbles

Item	**d_{gw}[5] (µm)**	**S_{gw}[6]**
Ground Corn	530	2.10
Mash	536	3.27
CCF[1]	1259	2.67
CCNF[2]	1646	2.15
FCF[3]	964	3.25
FCNF[4]	1041	2.68

[1] CCF = Coarse crumbles with fines retained.
[2] CCNF = Coarse crumbles with fines removed.
[3] FCF = Fine crumbles with fines retained.
[4] FCNF = Fine crumbles with fines removed.
[5] d_{gw} = Geometric average diameter.
[6] S_{gw} = Geometric standard deviation.

Table 4.3. Particle size distribution of the mash and crumbles

Particle Size Distribution, mm	**Mash**	**CCF**[1]	**CCNF**[2]	**FCF**[3]	**FCNF**[4]
> 1.18	8.39%	46.65%	55.59%	36.12%	35.42%
0.85 – 1.18	41.60%	29.96%	31.71%	35.85%	34.98%
0.30 – 0.84	24.33%	13.87%	9.17%	15.14%	18.45%
< 0.30	25.69%	9.51%	3.53%	12.89%	11.15%

[1] CCF = Coarse crumbles with fines retained.

[2] CCNF = Coarse crumbles with fines removed.

[3] FCF = Fine crumbles with fines retained.

[4] FCNF = Fine crumbles with fines removed.

Table 4.4. Effects of feed form on BW, ADG, ADFI, and FCR of broilers raised from 0 to 21 d

Growth Phase	**0 – 7**				**0 – 14**				**0 – 21**			
Item	**BW (g)**	**ADFI (g)**	**ADG (g)**	**FCR (g/g)**	**BW (g)**	**ADFI (g)**	**ADG (g)**	**FCR (g/g)**	**BW (g)**	**ADFI (g)**	**ADG (g)**	**FCR (g/g)**
Diet												
Mash	168^{c}	19.47^{c}	17.78^{c}	1.097^{a}	473^{c}	37.02^{b}	30.09^{b}	1.234^{a}	891^{c}	54.52^{c}	39.78^{c}	1.373^{a}
MP[1]	189^{b}	22.12^{ba}	20.75^{b}	1.066^{b}	555^{a}	41.96^{a}	35.70^{a}	1.181^{b}	1049^{a}	60.55^{a}	47.15^{a}	1.289^{b}
CCF[2]	199^{a}	23.35^{a}	22.27^{a}	1.049^{b}	553^{a}	41.47^{a}	35.76^{a}	1.160^{b}	1008^{b}	58.43^{b}	45.41^{ba}	1.288^{b}
CCNF[3]	199^{a}	23.24^{a}	22.20^{a}	1.048^{b}	561^{a}	41.41^{a}	36.39^{a}	1.139^{b}	1043^{a}	58.17^{b}	47.12^{a}	1.235^{c}
FCF[4]	197^{a}	23.41^{a}	21.91^{a}	1.069^{ba}	539^{b}	41.31^{a}	34.67^{a}	1.194^{b}	994^{b}	57.96^{b}	44.62^{b}	1.304^{b}
FCNF[5]	194^{ba}	22.74^{ba}	21.49^{ba}	1.059^{b}	547^{a}	41.28^{a}	35.96^{a}	1.149^{b}	1030^{a}	58.99^{b}	46.03^{ba}	1.287^{b}
SEM[6]	3.15	0.41	0.40	0.012	6.20	0.67	0.72	0.019	10.32	0.88	0.97	0.024
P Value	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	0.013	<0.0001	<0.0001	<0.0001	<0.0001

[1]MP = Micro-pellets; [2]CCF = Coarse crumbles with fines retained; [3] CCNF= Coarse crumbles with fines removed; [4]FCF = Fine crumbles with fines retained; [5]FCNF = Fine crumbles with fines removed.

[6]SEM = Standard error of the mean.

$^{a\text{-}c}$ Means in a column with different superscripts are significantly different at $P \leq 0.05$.

Table 4.5. Effects of feed form on relative gizzard weight (RGW) at 21 d

Treatment	**RGW (%)**
Mash	1.96^{a}
MP[1]	1.61^{c}
CCF[2]	1.58^{c}
CCNF[3]	1.60^{c}
FCF[4]	1.81^{b}
FCNF[5]	1.72^{b}
SEM[6]	0.021
P Value	<0.0001

[1] MP = Micro-pellets.

[2] CCF = Coarse crumbles with fines retained.

[3] CCNF= Coarse crumbles with fines removed.

[4] FCF = Fine crumbles with fines retained.

[5] FCNF = Fine crumbles with fines removed.

[6] SEM = Standard error of the mean.

[a-c] Means in a column with different superscripts are significantly different at $P \leq 0.05$.

Chapter 5 - Growth performance, intestinal morphometry, and blood serum parameters of broiler chickens fed diets containing increasing levels of wheat bran with or without exogenous enzyme supplementation

ABSTRACT

The objective of the study was to investigate the effects of enzyme supplementation on the growth performance, intestinal morphometry (villi height and crypt depth), and blood parameters of broilers fed diets containing increasing levels of wheat bran (WB). A total of 720 seven-day-old chicks were assigned to 1 of 8 treatments to obtain 15 chicks per pen and 6 replicates per treatment. A 2 x 4 factorial arrangement with 2 levels of enzymes (0 g/ton and 360 g/ton) and 4 levels of WB (0, 4, 8, and 12%) were used. The multi-enzyme supplement consisted of xylanase, β-glucanase and phytase (XGP). Four basal diets were manufactured and fed to grower (8 to 35 d) and finisher (36 to 42 d) birds either with or without XGP supplementation. BW and feed consumption were determined at 14, 28, and 42 d. ADG and ADFI were calculated. Mortality was checked daily, removed, weighed, and used to correct for FCR. Interactions ($P < 0.05$) were observed between level of WB and XGP for BW, ADG, and FCR at 42 d. Increasing the level of WB above 8% decreased BW and ADG and increased FCR of broilers but had no effect on ADFI. Additionally, interactions ($P < 0.05$) were observed for villi height (VH) and villi height to crypt depth ratio (VH:CD). The addition of XGP increased ($P < 0.01$) VH and VH:CD but had no effect on crypt depth. XGP increased ($P < 0.01$) HDCL but decreased RBC, HGB, and CHOL concentration of the blood. Increasing the level of WB increased HDCL of broilers. The results of the study indicated that feeding broilers WB and supplementing with

XGP resulted in similar growth performance and FCR as the control diets. The results also indicated that adding XGP to broiler diets increased VH, VH:CD, and HDCL but decreased RBC, HGB, and CHOL.

INTRODUCTION

The increasing demand for poultry products globally has resulted in the need for poultry producers to increase production to meet this demand. This has led to a corresponding increase in production inputs, including feed worldwide. Because poultry, like other livestock, competes with humans for food, the search for alternative ingredients and how to effectively utilize them has become increasingly important. Cereal by-products are alternative feed ingredients which could be used as energy sources for poultry and other livestock. The major wheat by-products available for use in poultry diets are wheat bran (WB), wheat shorts or brown shorts, and wheat middlings (Patrick and Schaible, 1981). Among these by-products, wheat bran and wheat middlings are the most extensively studied and have been utilized in animal feed formulations. These products are often diverted to animal feeds since they have little value in human food (Dale, 1996; Jaroni et al., 1999a; Ahmadi and Karimov, 2010).

Wheat bran is a by-product of dry milling of wheat to produce flour and consists of the outer layers (cuticle, pericarp, and seed coat) combined with small amounts of starchy endosperm of the wheat kernel (Hemery et al., 2007). It is one of the most popular food by-products used in poultry feed in developing countries. WB is a source of carbohydrates, protein, minerals, and vitamins. According to the NRC (1994), WB has less metabolizable energy (**ME**), but higher crude protein (**CP**) content compared to corn. Wheat bran has higher levels of non-starch polysaccharides (**NSP**; arabinoxylans; **AX**) which are indigestible by endogenous enzymes of monogastric animals including poultry. The NSPs in WB reduces the digestibility of other nutrients and subsequently, decreasing body weight gain (**BWG**) and feed efficiency. Additionally, soluble NSPs increase the viscosity the digesta in the small intestine (**SI**) and by so doing decrease the interactions between digestive enzymes and substrates. Ultimately there is a reduction in nutrient absorption and broiler performance (Choct and Annison, 1991; Almirall et al., 1995; Choct et al.,1995). Furthermore, the presence of NSP could lead to higher levels of gut microflora in the SI (Choct et al., 1996) resulting in excess fermentation which could reduce nutrient digestion and absorption (Choct et al., 1999).

The positive effects of exogenous enzyme supplementation of diets containing high levels of NSPs are well documented (Khattak et al., 2006; Slominski, 2011; Anadón et al., 2019). In poultry production the well-established enzymes are phytases and carbohydrases such as xylanases, amylases, β-glucanases. According to Anadón et al. (2019) feed enzymes can alter gastrointestinal tract (GIT) microbial ecology by reducing undigested substrates and anti-nutritional factors and producing oligosaccharides in situ from dietary NSP with potential prebiotic effects. Lu et al. (2013) reported that exogenous enzymes in poultry diets increase energy availability, reduce viscosity of gut contents, and improve nutrient absorption. Supplemental carbohydrases are reported to improve animal performance by reducing the negative effects of NSP through 1) decreasing fermentation in the small intestines (Kiarie et al., 2013), reducing viscosity problems associated with AX and β-glucans, and 2) eliminating the nutrient encapsulating effect of the cell wall (Masey O'Neill et al., 2014). Additionally, the addition of exogenous enzymes in broiler diets is reported to improve growth performance and feed efficiency (Coppedge et al., 2012). The improved performance is attributed to increased apparent metabolizable energy (**AME**), ileal digestibility of energy, and retention of dry matter (Meng and Slominski, 2005; Leslie et al., 2007).

The benefits of phytases, carbohydrases, and proteases on diets containing wheat by-products and other fibrous ingredients are well established (Cowieson, 2010; Anadón et al., 2019; Wickramasuriya et al., 2019). These enzymes improve the nutritional value of feed through several suggested modes of action (Anadón et al., 2019). Yang et al. (2017) reported that carbohydrases such as xylanases break down cell wall components (soluble and insoluble AX), increase crude protein (**CP**) and neutral detergent fiber (**NDF)** digestibility thus improving poultry performance. Amerah et al. (2017) suggested that amylase and protease increase nutrient digestibility, broiler growth performance and feed efficiency. The mode of action of β-glucanases involves breaking down NSPs in order to decrease the viscosity and increase nutrient digestibility and absorption in the ileum (Anadón et al., 2019). Multi-enzyme blends have been reported to have synergistic effects in diets that contain NSP. diets formulated for the different growth stages of broilers fed wheat by-products is limited. Therefore, the objective of the study was to investigate the effects of multi-enzyme supplementation on the growth performance, intestinal morphometry (villi height and crypt depth), and blood serum parameters of broilers fed diets containing increasing levels of wheat bran.

MATERIALS AND METHODS

The experiment was conducted at the Livestock and Poultry Research Center (**LIPREC**), University of Ghana (**UG**), Accra, Ghana. All experimental procedures were approved by the Animal Ethics Committee of College of Basic and Applied Sciences, UG, Legon, Accra, Ghana.

Dietary Treatments and Experimental Design

Treatment diets were fed to in the grower (8 to 35 d) and finisher (36 to 42 d) phases. For both grower and finisher diets, four basal diets were formulated with corn and SBM to contain increasing levels of WB. All diets were fed with or without a multi-enzyme blend that consisted of xylanase, β-glucanase, and phytase (Hi-Tek, Kimyevi Maddeler San. Ve Tic Ltd. Sti., Turkey). The enzyme was added to the diets based on the manufacturer's recommendations of 360g/ ton. The experimental design was a 2 x 4 factorial arrangement with 2 levels of enzymes (0 and 360 g/ton) and 4 levels of wheat bran (0, 4, 8, and 12%). The multi-enzyme provided a guaranteed minimum of xylanase (336,000 IXU), β-glucanase (150,000 TGU) and phytase (350,000 FTU). The treatments used for the study were: 0% wheat bran, no enzyme (WB0–), 0% wheat bran plus enzyme (WB0+), 4% wheat bran, no enzyme (WB4–), 4% wheat bran plus enzyme (WB4+), 8% wheat bran, no enzyme (WB8–), 8% wheat bran plus enzyme (WB8+), 12% wheat bran, no enzyme (WB12–), and 12% wheat bran plus enzyme (WB12+). The 0% WB diets (WB0– and WB0+) both met the nutritional requirements of broilers and served as the control diets.

Feed Formulation and Manufacturing

A common corn-SBM-based starter diet which contained no WB was manufactured and fed to all broiler starter chicks from 0 to 7 d of age. Treatment diets were formulated the grower (8 to 35 d) and finisher (36 to 42 d) growth phases. All diets were formulated to meet or exceed the nutritional requirements of broilers based on Cobb 500 standards (Cobb-Vantress Inc., Siloam Springs, AR). All feeds were manufactured at LIPREC, UG, Legon, Accra. The ingredient and nutrient composition of the common starter diet are presented in Table 5.1 whereas that of the grower and finisher diets are shown in Tables 5.2 and 5.3, respectively. The corn was ground using a hammermill (Kingdom Engineering Co. Ltd., Accra, Ghana) and the ingredients were mixed in a 2-ton vertical mixer. The dry ingredients and added liquid fat were mixed for 10 minutes. The multi-enzyme blend, XGP was added directly to the diets in the mixer according to the manufacturer's recommendations at 360g/ ton to each treatment containing the enzyme supplement.

Birds, Housing, and Management

All birds were fed from 0 to 42 d in three phases (starter: 0 to 7 d, grower: 8 to 35 d, and finisher: 36 to 42 d). The management of the broilers conformed to the guidelines of the Animal Ethics Committee of CBAS, UG, Legon, Ghana. One-day-old Cobb 500 broiler chicks obtained from a local hatchery in Accra, Ghana, were fed a common starter diet under similar brooding conditions for 7 d. At d 7, a total of 720 chicks were randomly allocated to 48 floor pens (1 m x 2 m: 2 m^2) with 15 birds per pen, resulting in a stocking density of 8 birds per m^2. The study utilized 8 treatments in the growth performance with 6 replicates and 15 birds per pen (90 chickens per treatment). New wood shavings were added to each of the 48 floor pens. A plastic tube feeder and a plastic drinker were provided in each pen. The temperature of the house was kept at 32ºC for the first 7 d and reduced gradually to 28ºC from 8 to 14 d of age. From d 15 to the end of the study, the temperature of the house was kept at ambient (25 to 26ºC). The lighting program provided 24 hours of light to all birds throughout the experimental period. Coccidiostat was administered orally between 5 to 7 d of age and repeated again between 15 to 19 d of age. The broilers were also vaccinated against Newcastle disease (Newcastle Disease, Cevac® New L, Ceva, France) and Infectious Bursal Disease (Gumboro) administered orally at 14 and 21 d respectively. After vaccination all the broilers received hydro-soluble vitamins (Maxi Vitaconc, Devine Agvet Ltd, Osun State, Nigeria) prepared by dissolving 1 g of vitamin in 1 liter of water and administered orally. All diets were fed in a mash form, and birds were allowed *ad-libitum* access to water and feed throughout the experimental period.

Growth Performance Measurement

Body weight (**BW**) and feed consumption were recorded by pen at weekly intervals on 14, 21, 28, 35, and 42 d by weighing broilers and feeders. The BW of the broilers and feed disappearance were used to calculate ADG, and ADFI. Mortality was checked daily, removed, weighed, recorded, and added to the total pen. The FCR was adjusted for mortality. The FCR was calculated by dividing the ADFI by the ADG of broilers.

Measurement of Blood Parameters

On 42 d, one bird with an average pen BW (8 broilers per treatment) was selected for the collection of blood. The blood sample from each bird was collected by holding the bird vertically and placing a 10 ml needle into either the tail artery or vein. The blood (about 3 ml) was collected into a 4 ml sample tube (Surgifield Medicals, Middlesex, England) that contained K_3 EDTA as the anticoagulant. For serum profile, about 3 ml of blood was collected into 5 ml serum-separator vacuum tubes (Surgifield Medicals, Middlesex, England). Samples collected were analysed the same day for blood serum parameters.

Blood serum parameters were analyzed using an Advia 120 (Siemens AG, Munich, Germany) analyzer. Parameters evaluated were red blood cells (**RBC**), white blood cells (**WBC**), aspartate aminotransferase (**AST**), alanine aminotransferase (**ALT**), hematocrit (**HCT**), mean corpuscular volume (**MCV**), mean corpuscular hemoglobin (**MCH**), cholesterol (**CHOL**) and high-density lipoprotein cholesterol (**HDLC**).

Intestinal Morphometry

On 42 d, one bird with an average pen BW (8 broilers per treatment) was selected for the intestinal morphometry. The bird was euthanized via cervical dislocation to collect tissues for morphometric measurement [villi height (**VH**) and crypt depth (**CD**)]. Within 10 minutes of slaughter, 4 to 6 cm segments of the terminal ileum were cut, rinsed in distilled water, and immediately placed in 10% formalin fixative solution for further morphometric analysis at the Noguchie Memorial Institute, University of Ghana, Accra, Ghana. The ileum was considered as the portion of the small intestine extending from the Meckel's diverticulum to a point extending 40 mm proximal to the ileo-cecal junction.

The samples were prepared according to the method outlined by Bejo (1990). Intestinal samples were removed from the fixative and sliced using a microtome blade to expose the cross- and length- sections of the intestine surface. They were then transferred into cassette cases and placed under running water for 30 minutes to wash off the fixative. The samples were dehydrated by dipping them into graded concentrations of ethanol (75, 80, 95, and 100%) for 20 minutes at a time and subsequently in chloroform for 30 minutes. Tissue samples were then infiltrated in molten wax at 60ºC and kept overnight. Subsequently, the embedded tissues were blocked and sectioned at an angle of 5º and a thickness of about 0.5 to 0.7 µm using a microtome (Model Bright 5040, Bright Instrument Company Ltd., Huntington, England). Sections obtained were gently spread on water at a temperature of 40ºC, fixed onto glass slides and heated until the samples were dry. Tissues were then deparaffinized in xylene hydrated through alcohol, stained with hematoxylin and counter stained with eosin. Tissue sections were finally dehydrated using 95% alcohol and mounted on 1.2 mm double frosted extra-thick micro slides with cover slips.

Both the VH and CD from the birds were measured with the aid of an optical microscope (Eclipse E600, Nikon Corp., Tokyo, Japan), camera (XC77E, Sony Corp., Tokyo, Japan), and an image analysis software (Visilog 5.2, Noesis, Courtaboeuf, France). The VH was measured as the distance between the crypt mouth and the tip of villi whereas the CD was measured as the distance between the basement membrane and the mouth of the crypt and were expressed as micrometers (µm). The villi height: crypt depth ratio (**VH: CD**) was calculated as the VH divided by the CD from the results obtained.

Economics of Production

The market cost of the major ingredients, vitamin TM premix, and the multi-enzyme at the time of the production of the experimental diets is presented in Table 21. These were used to calculate the cost of feed per kg in Ghana Cedi (GH₵). The cost of feed per ton (GH₵), total cost of feed consumed (GH₵), cost of feed per kilogram weight gain (GH₵) and cost savings (%) were calculated. One US Dollar was equivalent to Five Ghana Cedi at the time of the study.

Statistical Analysis

The experimental data were subjected to analysis of variance (ANOVA) using the GLIMMIX procedure of SAS 9.4 (SAS Institute, 1994). The statistical model included the effects of different inclusion levels of wheat bran, multi-enzyme addition, and their interactions on the growth performance, intestinal morphometry, and blood serum parameters of broilers. Linear and quadratic contrasts were included in the model to compare the effects of increasing levels of WB on the response parameters. Mean differences were considered significant at ($P \leq 0.05$) using Tukey's test.

RESULTS

Growth Performance

Mortality during the study was 5.2% with no dietary treatment effects. The WB had a dry matter (**DM**) content of 89.7% and also contained a CP 10.8%, NDF 35.2%, and ADF 11.7% on DM basis. The influence of increasing levels of WB with or without XGP on growth performance of broilers is presented in Tables 16, 17, and 18. Overall there was a significant interaction ($P = 0.032$) between the level of WB and XGP on BW of broilers at 42 d of age.

There were no interactions ($P > 0.05$) for BW at 14, and 28 d of age. However, there was linear decrease in BW of broilers as the level of WB was increased in the diets at 14 ($P = 0.003$) and 28 ($P = 0.02$) d. In addition, XGP had no effects on BW of broilers at 14, and 28 d of age. Also, no quadratic response was observed at 14 ($P = 0.88$), and 28 ($P = 0.08$) d of age.

There was no significant interaction between the level of WB and XGP on ADFI at 14, 28, and 42 d of age. There were no significant differences on ADFI at 14, 28 d of age for broilers fed the different treatment diets. The inclusion level of WB and addition of XGP to the diets had no effects on ADFI throughout the study. Additionally, no linear or quadratic response were observed on ADFI at 14, 28, and 42 d of age for the different levels of WB in the diets. Overall the ADFI was not affected by dietary treatments. There were no interactions between the level of WB and XGP on ADG at 14 ($P = 0.21$), and 28 ($P = 0.53$) d of age. There was a linear ($P = 0.003$) decrease of ADG as the inclusion level of WB increased at 14 d of age. There were no linear or quadratic response on ADG at 28 d of age. However, the increase ($P = 0.03$) in the level of WB tended to decrease ADG at 28 d of age. There was an interaction ($P = 0.04$) between the level of WB and XGP on the overall ADG at 42 d of age. Additionally, there was a linear decrease on the overall ADG as the level of WB was increased in the diets at 42 d of age. However, no quadratic response was observed on the level of WB at 42 d of age. A significant interaction was observed between the level of WB and XGP for FCR at 14 ($P = 0.03$), and 28 ($P = 0.001$) d of age. Additionally, there was a linear increase in FCR as the level of WB was increased in the diets at 14 ($P = 0.001$) and 28 ($P = 0.001$) d of age. A quadratic response was also observed for the level of WB at 28 d of age. There was no interaction ($P = 0.70$) between the level of WB and XGP on the overall FCR at 42 d of age. The level of WB in the diets influenced the overall FCR but the addition of XGP had no effect on the overall FCR. Additionally, there was a linear increase in the overall FCR (*P* = 0.003) of broilers at 42 d.

Intestinal Morphometric Measurements

As shown in Table 19, significant interactions (*P* = 0.04) were observed between the level of WB and XGP for the intestinal VH (*P* = 0.04) and VH: CD (*P* = 0.04) of broilers. However, no interaction (*P* = 0.71) was observed between the level of WB and XGP on the intestinal CD of broilers. In addition, there were linear (*P* = 0.004) increase in VH in response to increasing levels of WB in the diets. and VH: CD of broilers. There was no linear or quadratic response on the intestinal CD of broilers.

Measurement of Blood Serum Parameters

The blood serum parameters measured are presented in Table 20. There were no interactions (*P* > 0.05) between the level of WB and XGP on red blood cells, white blood cells, hemoglobin, aspartate aminotransferase, alanine aminotransferase, cholesterol, hematocrit, mean corpuscular volume, and mean cell hemoglobin of broilers. However, interaction (*P* = 0.03) was observed between the level of WB and XGP on high-density lipoprotein cholesterol. There was a linear (P < 0.001) increase in HDCL as the level of WB was increased in the diets. The addition of WB affected only the HDLC concentration of the blood. In addition, there was no linear or quadratic (*P* > 0.05) response on RBC, WBC, HGB, AST, ALT, CHOL, HCT, MCV, and MCH for the inclusion level of WB. The addition of enzymes decreased RBC, WBC, HGB, CHOL, and HCT. However, no response to supplemental enzyme was observed for AST, ALT, MCV, and MCH.

Economics of Production

Table 21 shows the economics of including WB or XGP on the cost of feed. From the results obtained there was no significant difference in the feed cost per gain of broilers. This indicates broiler diet could be formulated with up to 12% of WB without affecting feed cost per gain.

DISCUSSION

Wheat bran is one of the most important by-product ingredients which can be used as an energy supplement to replace some percentage of corn in broiler diets. According to Leeson and Summers (2005), wheat bran possess growth promoting factors which is associated with the modification of bacterial population in the digestive tract of broilers and other poultry birds. However, WB contains relatively high amount of NSPs which are indigestible by the endogenous enzymes broilers which could negatively affect bird performance. Therefore, exogenous enzymes are supplemented to improve the digestive capacity of especially young poultry to improve digestion efficiency of NSP diets. The benefits of improving nutrient digestibility, availability, and bird performance with the supplementation of exogenous enzyme in poultry nutrition is well documented (Bedford and Morgan, 1996; Kiarie et al., 2013; Anadón et al., 2019). Using appropriate enzyme types improve the nutritional value of feed and efficiency of digestion thus improving animal performance. Multi-enzyme supplementation could provide a more appropriate means of improving nutrient utilization in livestock or poultry (Cowieson et al., 2006; Wickramasuriya et al., 2019). According to Meng (2005) and Francesch and Geraert (2009) the addition of enzyme multi-enzyme complex to broiler diets resulted in the release of metabolizable energy (**ME**) from otherwise unavailable sugars from NSP and P of phytate.

The results of the study showed that the inclusion of WB did not negatively affect performance. On d 14 and 28, no significant interactions were observed between the levels of WB and XGP on BW of broilers. However, there was a linear decrease in BW in response to increasing levels of WB. This indicated that increasing the level of WB in the diets resulted in the decline in BW of broilers. The BW of broilers were not affected by the including enzyme in the diet at 14 d. In accordance with the current study, Ali et al. (2008) reported no adverse effects of wheat bran on BW of broilers at 28 d. However, the inclusion of 3% WB in the diet of broiler starter from 7 to 14 d resulted in decreased BW (Ali et al., 2008). Kong and Adeola (2014) reported that the energy value in wheat bran is better utilized by broilers when supplemented with enzyme products containing carbohydrase due to increased AMEn. The ADFI at 14 and 28 d of age was affected dietary WB level, enzyme addition or their interaction. Feeding 12% diet did not negatively affected ADFI of broilers.

The overall BW of broilers from the current study showed significant interaction between the level of WB and XGP at 42 d. Generally, the interactions between high fiber diets and exogenous enzymes is expected to improve growth performance of animals by breaking down plant cell wall materials and improve the nutrient utilization of feed (Bedford and Partridge, 2001; Amerah et al., 2017; Anadón et al., 2019). This is contrary to the results obtained from the current study where interaction resulted in inconsistent performance of broilers. There was both linear and quadratic response on BW of broilers due to the inclusion of WB in diets. Increasing the level of WB deceased BW of broilers. The addition of XGP had no effects on the overall BW of broilers. On the contrary, Yu et al. (2007) and Amerah et al. (2017) reported in improvement in growth performance of broiler fed diets supplemented with multi-enzyme. The application of multi-enzyme could lead to either additive, sub-additive, or synergistic effects on nutrient utilization and livestock growth performance (Ravindran et al., 1999; Juanpere et al., 2005). Other researchers reported inconsistent results in corn- and sorghum-based diets with supplemental enzyme (Cowieson, 2005; Cowieson et al., 2006a, b).

Wickramasuriya et al. (2019) investigated the effect of dietary Multi-Carbohydrase (MC) supplementation on growth performance, blood metabolites, visceral organ weights, gut morphology, and nutrient digestibility in broiler chickens. The authors fed four treatment diets made up of a positive control, a negative control, a PC with MC (MC; Superzyme-CSTM; 0.05%); and a NC with MC to broiler chickens in a 35-d study period. In the study, birds fed corn-SBM diet containing wheat, and wheat bran supplemented with MC showed significantly higher growth performance (BW and FCR) compared to birds fed diets without MC. This is contrary to the current study where multi-enzyme supplementation had no effect on BW of broilers.

There was no interaction between the level of WB and XGP on overall ADFI of broilers at 42 d. There were no linear or quadratic response on the level of WB for ADFI indicating that increasing the level of WB in the diet did not affect feed intake negatively. Additionally, XGP supplementation had no effects on the overall ADFI. This agreed with Tahir et al. (2005) who reported no response on feed intake with carbohydrases (hemicellulase, cellulase, and their combination) supplementation. On the contrary, Cowieson and Ravindran (2008b) reported increased feed intake in broilers fed diets supplemented with a mixture of xylanase, amylase, and protease. Wang et al. (2005) also reported no response in feed intake of broilers fed wheat-based diets supplemented with enzyme at 42 d of age. Similarly, Nian et al. (2011) found no significant difference in feed intake in broilers fed a wheat-based diet and supplemented with exogenous xylanase.

Commercially exogenous enzymes have been used over the years to improve nutrient digestibility, growth performance, and feed efficiency in both wheat-SBM and corn-SBM based diets. The 42 d FCR observed in this study showed no interaction between the level of WB and XGP multi-enzyme for broilers fed the different treatment diets. From the results obtained XGP had no effect on the overall FCR of broilers at 42 d. This is contrary to the results obtained by Cowieson and Ravindran (2008) where improvements in FCR was reported with the supplementation of carbohydrases to the broiler diets. Amerah et al. (2017) investigated the effect of exogenous xylanase, amylase, and protease (X, A, and P) as single or combined activities on nutrient digestibility and growth performance of broilers fed corn-based diets. From the study, xylanase supplementation or in combination with amylase and protease improved the FCR compared to the negative control (**NC**) diet over the entire experimental period. The improved performance was attributed to increased access of endogenous and exogenous enzymes to protein and starch within the endosperm cell Cowieson (2005) by degrading the highly branched insoluble arabinoxylans in the cell wall (Chesson, 2001). Similarly, Cowieson et al. (2010) observed that the supplementation of Corn-SBM based broiler diets with different doses of xylanase and glucanase, and their combination, improved FCR and ideal nutrient digestibility.

Research has indicated that exogenous enzyme supplementation affects the intestinal morphology of broilers fed diets containing NSPs or decrease the fermentation in the small intestines due to high NSP diets (Choct et al., 1996). From the study, interactions between the level of WB and XGP increased villi height and VH: CD but had no effect on crypt depth. Feeding WB diets linearly increased VH and VH: CD of broilers. The supplementation of the diets with XGP significantly increased the villi height and VH: CD. However, there was no response of CD to both the level of WB and XGP supplementation. The inclusion of moderate levels of diets containing NSP is recognized to increase the gut size, length, volume, and morphological structure (villi height and crypt depth) of poultry. A well-developed intestinal morphological structure improves the efficiency of nutrient digestion and absorption in broilers. From the study, villi height increased in response to increase in WB level of the diets. Yamauchi and Tarachai (2000) demonstrated a positive correlation between the villi height and nutrient absorption. Increased nutrient absorption results in improvement in growth performance and feed efficiency. The VH: CD is considered as a useful measure of the potential digestive capacity of the small intestine of monogastric animals (Jha et al., 2019). Therefore, a high VH: CD results in an improvement in digestion and absorption of nutrients in broilers. However, Mathlouthi et al. (2002) reported a reduction in villi height, width, and surface area in broilers fed the rye-based compared to corn-based diets resulting in poorer feed intake, weight gain, and feed efficiency. The decreased in broiler performance was attributed to the reduced capacity of the villi to absorb nutrients. Also, the authors reported that the inclusion of xylanase and β-glucanase increased the VH and VH: CD of the small intestine leading to improvement in nutrient absorption. This is consistent with the results of the current study where the addition of the XGP multi-enzyme to the basal diets increased VH and VH: CD of the ileum. Other researchers have also reported Luo et al. (2009) reported increased villi height of the duodenum, jejunum and ileum with xylanase supplementation. The results of the study indicated that increasing the level of WB in the diets increased both the villi height and villi height to crypt depth ratio of broilers.

From the results obtained from the study, no interactions were observed between the level of WB and XGP for RBC, WBC, HGB, ALT, AST, CHOL, HCT, MCV, and MCH. However, significant WB x XGP was observed for HDCL of broilers. The main effects of WB showed no linear or quadratic response on RBC, WBC, HGB, ALT, AST, CHOL, HCT, MCV, and MCH.

On the other hand, the inclusion of WB in the diets linearly increased the HDCL concentration of the blood. In addition, the concentration of blood HDCL increased with the addition of XGP to the diets. The supplementation of XGP decreased the concentration of RBC, HGB, and CHOL. Research has indicated that high levels of dietary CHOL as a result of low dietary fiber content or enzyme supplementation could increase the absorption of CHOL leading to increase in plasma CHOL levels (Sutton et al., 1985; Mancini and Parillo 1991; Pettersson and Aman, 1992). This contradicted the results obtained from the current study where WB had no effect on the concentration of CHOL in the blood. In agreement with the current study, Zarghi et al. (2010) reported no significant difference in CHOL concentration of broilers fed diets containing different levels of triticale, an NSP rich diet. The addition of the multi-enzyme significantly reduced the CHOL concentration of the blood. According to Després et al. (2000) high serum HDLC concentrations may prevent coronary heart diseases in humans. In broilers higher HDCL could improve the cardiovascular function, thus reducing mortality and improving on the meat quality. Moreover, the supplementation of XGP further increased the HDCL concentration of the blood of broilers. This agreed with Hajati et al. (2009) who observed that enzyme addition increased HDCL and triglycerides at 42 d. The results of the study indicated that the addition of XGP to diets WB containing WB increased HDCL concentration of the blood thus reducing the risks of cardiovascular diseases which causes high mortality in broilers. The results also indicated that XGP decreased RBC, HGB and CHOL.

From the study including 4 to 12% of WB in the basal diets reduced total feed cost per ton, but feed cost per kg of gain was not significantly different across treatments. WB is abundant in developing countries such as Ghana and is useful as an energy supplement in situations where corn is unavailable. Therefore, broiler diets could be formulated with up to 12% of WB, without negatively affecting performance and incurring an extra cost.

CONCLUSION AND APPLICATION

1. Feeding broilers 4% wheat bran and supplemented with XGP multi-enzyme increased growth performance and improved feed efficiency of broilers.
2. Feeding diets containing 8% wheat bran with or without exogenous enzyme resulted in similar performance as the 0% diets and could be utilized to reduce feed cost.
3. Feeding 12% WB diets did not impair feed intake but decreased BW and increased FCR compared to the other diets.
4. Both enzyme supplementation and inclusion of WB increased the villi height and villi height to crypt depth ratio which improved nutrient digestion and absorption but had no effect on crypt depth.
5. Increasing the level of WB in the diets increased HDCL but had no effects on the other blood serum parameters measured.
6. Addition of exogenous enzyme decreased RBC, HGB, CHOL but increased HDCL concentrations of the blood of broilers.

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Table 5.1. Composition of common broiler starter diet[1]

Ingredient, %	**Starter**
Corn	51.78
SBM	35.05
Soy oil	5.50
L-Lysine HCL	0.20
DL-Methionine	0.32
L-Threonine	0.25
Dicalcium Phosphate	1.10
Limestone	1.25
Salt	0.40
Vitamin TM Premix[2]	0.40
Total	100.00
Calculated Nutrient Composition	
ME, kcal/kg	3010.00
CP, %	21.50
Total Lysine, %	1.32
Digestible Lysine, %	1.18
Digestible Methionine, %	0.46
Digestible Threonine, %	0.78
Dig. Meth + Cys, %	0.89
Ca, %	0.90
Avail P, %	0.45
Analyzed Nutrient Composition	
DM	87.66
Crude Protein	21.62
Crude Fiber	2.70
Ether Extract	8.04
ADF	5.21
NDF	9.40

[1] Diet: A common starter diet was formulated for all 1-day-old chicks and fed from 0 to 7 d.

[2] Vitamin TM Premix provided per kg of diet: Vitamin A, 12,000,000IU; Vitamin D3, 2,000,000 IU; Vitamin E, 10,000 mg; Vitamin K3, 1,500 mg; Vitamin B1, 1,500 mg; Vitamin B2, 4000 mg; Vitamin B6, 1,500 mg; Vitamin B12, 15 mg; Pantothenic Acid, 8000 mg; Nicotinic Acid, 20,000 mg; Folic Acid, 500 mg; Biotin, 150 mg; Choline Chloride, 120,000 mg; Iron, 40,000 mg, Manganese, 60,000 mg; Copper, 6000 mg; Zinc, 50,000 mg; Iodate, 2,000 mg; Cobalt, 200 mg; Selenium, 150 mg.

Table 5.2. Composition of dietary treatments fed to cobb 500 broiler growers from 8 to 35 d

	WB (%) of Grower Diets[1]			
Diets	**0%**	**4%**	**8%**	**12%**
Ingredient		**(%)**		
Corn	58.88	56.04	52.04	50.65
SBM	31.30	29.60	29.60	26.50
Wheat Bran	0.00	4.00	8.00	12.00
Soy oil	6.00	6.51	6.51	7.00
L-Lysine HCL	0.27	0.30	0.30	0.37
DL-Methionine	0.40	0.40	0.40	0.42
Dicalcium Phosphate	1.10	1.10	1.10	1.10
Limestone	1.25	1.25	1.25	1.25
Salt	0.40	0.40	0.40	0.40
Vitamin TM Premix[2]	0.40	0.40	0.40	0.40
Hi-Zyme Exp Premix[3]	-	-	-	-
Total	100.00	100.00	100.00	100.00
Calculated Nutrient Composition				
ME, kcal/kg	3090.00	3090.00	3090.00	3090.00
CP, %	19.20	19.20	19.20	19.20
Total Lysine, %	1.20	1.20	1.20	1.20
Dig. Lysine, %	1.06	1.06	1.06	1.06
Dig. Methionine, %	0.43	0.43	0.43	0.43
Dig. Threonine, %	0.70	0.70	0.70	0.70
Dig. Meth + Cys, %	0.81	0.81	0.81	0.81
Ca, %	0.90	0.90	0.90	0.90
Avail P, %	0.43	0.43	0.43	0.43
Analyzed Results				
DM	89.07	89.06	89.10	89.03
Crude Protein	19.51	19.53	19.47	19.45
Crude Fiber	2.83	3.05	3.24	3.91
Ether Extract	8.58	9.13	9.15	9.37
ADF	4.62	4.41	4.01	5.33
NDF	9.60	9.91	10.23	11.35

[1] Grower diets: Basal diets formulated to contain increasing levels of WB without addition of enzyme. Exogenous enzyme (Hi-Zyme Exp Premix) added on top of each diet at 360 g/ton to obtain the dietary treatments with enzymes.

[2] Vitamin TM Premix provided per kg of diet: Vitamin A, 12,000,000IU; Vitamin D3, 2,000,000 IU; Vitamin E, 10,000 mg; Vitamin K3, 1,500 mg; Vitamin B1, 1,500 mg; Vitamin B2, 4000 mg; Vitamin B6, 1,500 mg; Vitamin B12, 15 mg; Pantothenic Acid, 8000 mg; Nicotinic Acid, 20,000 mg; Folic Acid, 500 mg; Biotin, 150 mg; Choline Chloride, 120,000 mg; Iron, 40,000 mg, Manganese, 60,000 mg; Copper, 6000 mg; Zinc, 50,000 mg; Iodate, 2,000 mg; Cobalt, 200 mg; Selenium, 150 mg.

[3] Hi-Zyme Exp Premix: contained endo-1,4-β-Xylanase (336,000 IXU), endo-1,4-β-Glucanase (150,000 TGU) and Phytase (350,000 FTU).

Table 5.3. Composition of dietary treatments fed to cobb 500 broiler finishers from 36 to 42 d

	WB (%) of Finisher Diets			
Diets	**0%**	**4%**	**8%**	**12%**
Ingredient		**(%)**		
Corn	62.68	59.39	56.21	52.82
SBM	27.50	26.50	25.70	24.50
Wheat Bran	0.00	4.00	8.00	12.00
Soy oil	6.20	6.51	6.51	7.00
L-Lysine HCL	0.24	0.22	0.20	0.30
DL-Methionine	0.23	0.23	0.23	0.23
Dicalcium Phosphate	1.10	1.10	1.10	1.10
Limestone	1.25	1.25	1.25	1.25
Salt	0.40	0.40	0.40	0.40
Vitamin TM Premix[2]	0.40	0.40	0.40	0.40
Hi-Zyme Exp Premix[3]	-	-	-	-
Total	100.00	100.00	100.00	100.00
Calculated Nutrient Composition				
ME, kcal/kg	3,169.00	3,169.00	3,169.00	3,169.00
CP, %	18.30	18.30	18.30	18.30
Total Lysine, %	1.06	1.06	1.06	1.06
Dig. Lysine, %	0.96	0.96	0.96	0.96
Dig. Methionine, %	0.40	0.40	0.40	0.40
Dig. Threonine, %	0.66	0.66	0.66	0.66
Dig. Meth + Cys, %	0.75	0.75	0.75	0.75
Ca, %	0.77	0.77	0.77	0.77
Avail P, %	0.39	0.39	0.39	0.39
Analyzed Nutrient Composition				
DM	89.13	89.11	89.14	89.13
Crude Protein	18.61	18.45	18.47	18.48
Crude Fiber	2.85	2.89	3.34	3.88
Ether Extract	7.91	9.21	9.24	9.56
ADF	4.58	4.21	3.89	5.26
NDF	9.71	9.80	10.31	11.33

[1] Finisher diets: Basal diets formulated to contain increasing levels of WB without addition of enzymes. Exogenous enzyme (Hi-Zyme Exp Premix) added on top of each diet at 360 g/ton to obtain the dietary treatments with enzymes.

[2] Vitamin TM Premix provided per kg of diet: Vitamin A, 12,000,000IU; Vitamin D3, 2,000,000 IU; Vitamin E, 10,000 mg; Vitamin K3, 1,500 mg; Vitamin B1, 1,500 mg; Vitamin B2, 4000 mg; Vitamin B6, 1,500 mg; Vitamin B12, 15 mg; Pantothenic Acid, 8000 mg; Nicotinic Acid, 20,000 mg; Folic Acid, 500 mg; Biotin, 150 mg; Choline Chloride, 120,000 mg; Iron, 40,000 mg, Manganese, 60,000 mg; Copper, 6000 mg; Zinc, 50,000 mg; Iodate, 2,000 mg; Cobalt, 200 mg; Selenium, 150 mg.

[3] Hi-Zyme Exp Premix: contained endo-1,4-β-Xylanase (336,000 IXU), endo-1,4-β-Glucanase (150,000 TGU) and Phytase (350,000 FTU).

Table 5.4. Body weight of broilers fed increasing levels of wheat bran with or without exogenous enzyme supplementation from 14 to 42 d

Item	**Body Weight, g/ Bird per Period, d**			
Diet (% WB)	**Enzyme**	**14 d**	**28 d**	**42 d**
0%	-	427	1421	2702^{bac}
0%	+	444	1395	2680^{bac}
4%	-	438	1402	2706^{bac}
4%	+	442	1457	2807^{a}
8%	-	416	1409	2716^{ba}
8%	+	434	1387	2575^{bac}
12%	-	415	1352	2467^{c}
12%	+	409	1337	2553^{bc}
Pooled SEM		8.59	21.01	38.54
Main Effects				
Wheat Bran				
0%		436	1408	2691
4%		440	1429	2756
8%		425	1398	2645
12%		412	1344	2510
SEM		6.08	18.05	38.55
Enzyme				
	-	429	1396	2648
	+	428	1394	2653
SEM		4.30	14.86	27.26
Source of Variation			**P-value**	
Wheat Bran		0.01	0.04	0.001
Enzyme		0.88	0.94	0.88
Wheat Bran*Enzyme		0.23	0.49	0.032
Linear		0.03	0.02	0.001
Quadratic		0.88	0.08	0.005

$^{a-c}$ Means within a column with different superscript are significantly different at ($P < 0.05$).

SEM = Standard error of the mean.

Table 5.5. Effects of Level of wheat bran inclusion and enzyme supplementation on ADFI, and ADG of broilers from 14 to 42 d

Item		**Growth Parameters**					
Growth Parameter		**ADFI, g/bird**			**ADG, g/bird**		
Diet (% WB)	**Enzyme**	**14 d**	**28 d**	**42 d**	**14 d**	**28 d**	**42 d**
0%	-	50.5	83.6	149.0	38.6	59.3	71.5^{ba}
0%	+	52.2	84.0	158.2	41.0	58.9	70.4^{bac}
4%	-	52.5	88.1	161.9	39.8	59.4	69.9^{bac}
4%	+	51.3	84.4	155.3	40.5	61.6	74.2^{a}
8%	-	49.9	83.6	164.7	39.1	59.6	70.8^{ba}
8%	+	49.2	86.0	155.1	36.2	58.2	68.6^{bc}
12%	-	49.9	86.4	143.5	36.9	56.7	65.3^{c}
12%	+	51.1	87.2	153.7	35.3	55.7	66.8^{bc}
Pooled SEM		1.44	1.90	7.09	1.34	1.38	1.16
Main Effects							
Wheat Bran							
0%		51.3	83.8	153.6	39.77	59.1	70.9
4%		51.9	86.2	158.6	40.11	60.5	72.0
8%		49.5	84.8	159.9	37.61	58.9	69.7
12%		50.5	86.8	148.6	36.11	56.2	66.1
SEM		1.02	1.35	5.01	0.94	0.97	0.58
Enzyme							
	-	50.7	85.4	154.8	38.6	58.7	69.3
	+	51.0	85.4	155.6	38.2	58.6	70.0
SEM		0.72	0.95	7.09	0.57	0.69	0.58
Source of Variation					**P Value**		
Wheat Bran		0.40	0.41	0.38	0.72	0.03	<0.001
Enzyme		0.80	0.98	0.87	0.01	0.88	0.45
Wheat Bran*Enzyme		0.71	0.42	0.37	0.21	0.53	0.036
Linear		0.30	0.30	0.21	0.003	0.40	0.02
Quadratic		0.82	0.77	0.10	0.39	0.13	0.08

$^{a-c}$ Means within a column with different superscript are significantly different at ($P < 0.05$).

SEM= Standard error of the means.

Table 5.6. Effects of level of wheat bran and enzyme supplementation on FCR of broilers from 7 to 42 d.

Item	**FCR, g/ g per Bird per Period, d**			
Diet (% WB)	**Enzyme**	**14 d**	**28 d**	**42 d**
0%	-	1.312^{b}	1.411^{dc}	1.814
0%	+	1.277^{b}	1.429^{bdc}	1.801
4%	-	1.319^{b}	1.485^{bac}	1.839
4%	+	1.274^{b}	1.368^{d}	1.688
8%	-	1.281^{b}	1.405^{dc}	1.952
8%	+	1.361^{ba}	1.480^{bac}	1.877
12%	-	1.356^{ba}	1.524^{ba}	1.984
12%	+	1.452^{a}	1.566^{a}	1.904
Pooled SEM		0.03	0.02	0.06
Main Effects				
Wheat Bran				
0%		1.294	1.420	1.808
4%		1.296	1.427	1.764
8%		1.321	1.443	1.915
12%		1.404	1.545	1.944
SEM		0.02	0.02	0.04
Enzyme				
	-	1.317	1.456	1.92^{a}
	+	1.341	1.461	1.82^{b}
SEM		0.01	0.012	0.03
Source of Variation			**P-value**	
Wheat Bran		0.002	<0.001	0.01
Enzyme		0.31	0.78	0.03
Wheat Bran*Enzyme		0.02	0.001	0.60
Linear		0.001	0.001	0.003
Quadratic		0.07	0.01	0.31

$^{a\text{-}d}$ Means within a column with different superscript are significantly different at ($P < 0.05$)

SEM= Standard error of the means.

Table 5.7. Effects of level of wheat bran and enzyme supplementation on villi height, crypt depth, and villi height: crypt depth (VH:CD) of broilers

Item		**Morphometric Measurements**		
Diet (% WB)	**Enzyme**	**Villi Height (µm)**	**Crypt Depth (µm)**	**VH:CD**
0%	-	523^{d}	117	4.48^{d}
0%	+	599^{bdac}	115	5.24^{bdac}
4%	-	528^{dc}	110	4.79^{dc}
4%	+	711^{a}	111	6.39^{ba}
8%	-	571^{bdc}	113	5.06^{bdc}
8%	+	668^{ba}	110	6.26^{ba}
12%	-	652^{bdac}	112	5.85^{bac}
12%	+	660^{bac}	103	6.48^{a}
Pooled SEM		29.72	4.12	0.30
Main Effects				
Wheat Bran				
0%		561	116	4.86
4%		619	111	5.59
8%		620	111	5.66
12%		656	107	6.16
SEM		21.02	2.92	0.21
Enzyme				
-		569^{b}	113	5.05b
+		659^{a}	109	6.09a
SEM		14.86	2.06	0.15
Source of Variation		**P-Value**		
Wheat Bran		0.023	0.22	0.001
Enzyme		<0.001	0.20	<0.001
Wheat Bran*Enzyme		0.044	0.71	0.036
Linear		0.004	0.05	0.001
Quadratic		0.60	0.8	0.60

$^{a-d}$ Means within a column with different superscript are significantly different at ($P < 0.05$).
SEM= Standard error of the means.

Table 5.8. Effects of level of wheat bran and enzyme supplementation on hematological measurements of broilers.

		Blood Parameters									
Diet (% WB)	**Enzyme**	**RBC**	**WBC**	**HGB**	**AST**	**ALT**	**CHOL**	**HDLC**	**HCT**	**MCV**	**MCH**
0%	-	13.24	2.88	13.47	636	146	4.32	1.44^{f}	30.28	102	48.51
0%	+	12.69	2.61	12.52	644	150	3.65	2.18^{dc}	29.01	103	48.42
4%	-	12.74	2.70	13.17	636	155	4.31	1.47^{fe}	30.75	110	49.30
4%	+	12.05	2.70	13.27	546	150	4.00	2.37^{bc}	28.47	105	49.12
8%	-	12.94	2.86	13.30	718	153	3.99	1.85^{de}	30.95	105	48.52
8%	+	11.76	2.61	12.60	586	150	3.95	2.68^{ba}	29.36	105	47.53
12%	-	12.77	2.77	13.08	595	154	4.21	2.30^{bc}	28.80	101	47.85
12%	+	11.90	2.83	12.10	498	152	3.56	2.87^{a}	28.82	103	48.17
Pooled SEM		0.42	0.083	0.33	56.28	2.35	0.21	0.09	0.89	2.31	0.65
Main Effects											
Wheat Bran											
0%		12.96	2.75	12.99	640	148	3.98	1.81	29.50	103	48.47
4%		12.39	2.70	13.22	591	152	4.15	1.94	29.61	107	49.21
8%		12.35	2.73	12.95	652	152	3.97	2.26	30.15	105	48.03
12%		12.34	2.80	12.59	546	153	3.88	2.58	28.81	102	48.00
SEM		0.29	0.058	0.23	39.80	1.66	0.15	0.06	0.63	1.63	0.46
Enzyme											
-		12.92^{a}	2.80a	13.25^{a}	646	152	4.21^{a}	1.76^{b}	30.13^{a}	105	48.55
+		12.10^{b}	2.67b	12.62^{b}	568	151	3.79^{b}	2.52^{a}	28.82^{b}	104	48.31
SEM		0.21	0.04	0.17	28.14	1.17	0.10	0.05	0.45	1.15	0.32
Source of Variation						**P-Value**					
Wheat Bran		0.41	0.54	0.32	0.24	0.21	0.62	<0.001	0.52	0.12	0.46
Enzyme		0.01	0.04	0.01	0.06	0.35	0.01	<0.001	0.04	0.59	0.32
Wheat Bran*Enzyme		0.83	0.17	0.34	0.54	0.25	0.34	0.35	0.48	0.39	0.62
Linear		0.14	0.45	0.17	0.22	0.06	0.46	<0.001	0.64	0.66	0.25
Quadratic		0.45	0.25	0.21	0.50	0.55	0.41	0.16	0.32	0.03	0.66

$^{a-f}$ Means within a column with different superscript are significantly different at ($P < 0.05$).

SEM= Standard error of the means.

Chapter 6 - Findings of the Research

The current dissertation evaluated the effects of feed processing (pelleting and crumbling) and the inclusion of wheat bran with or without exogenous multi-enzyme supplementation on broiler performance. The major factors examined included the physical feed form (crumbles vs mash diet), the inclusion level of wheat bran, and supplemental multi-enzyme. The research also investigated techniques to stimulate early chick growth by increasing feed intake through feeding crumbled diets.

The major finding of the research was the improvement in the growth performance of broilers fed crumbled diets compared to feeding a mash diet. Feeding crumbled diet for a minimum of 7 d to broiler chicks increased the feed intake, body weight, and improved feed conversion ratio. Additionally, increasing the length of time of feeding crumbles up to 21 d further improved broiler performance. In broiler production systems, feeding crumbles generally increases feed intake, body weight, and improves feed conversion compared to feeding a mash diet. The results of the research reported in this dissertation indicated that feeding quality crumbles by removing the fines from the crumbles further increased the growth performance of broilers fed the crumbled diets.

The use of alternative fibrous ingredients could replace some of the corn and SBM in broiler diets, thus reducing the cost of feed. The results of the third study suggested that including up to 8% of wheat bran (WB) in broiler diets with or without multi-enzyme supplementation resulted in similar growth performance as the conventional corn-SBM diet. Additionally, including12% WB in a broiler diet with multi-enzyme improved the growth performance and intestinal morphometry of broilers and resulted in similar performance as the 0% WB diets. The research data further suggested that broiler diets could be formulated with up to 12% WB without compromising on performance. However, further research is required to determine the type of multi-enzyme that will lead to the best performance and increase in the return of investment.