The Effects of Feed Ingredients and Manufacture Technique on Pellet Quality and Phytase Inclusion Levels on Broiler Performance

Tyler R. Rigby

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The Effects of Feed Ingredients and Manufacture Technique on Pellet Quality and Phytase Inclusion Levels on Broiler Performance

Tyler R. Rigby

Thesis submitted
to the Davis College of Agriculture, Natural Resources and Design
at West Virginia University

in partial fulfillment of the requirements for the degree of

Master of Science in
Animal and Food Science

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Keywords: corn DDGS, pellet quality, mixer-added fat, dicalcium phosphate, steam conditioning temperature, moisture content, phytase, environmental effects, broiler performance

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ABSTRACT

The Effects of Feed Ingredients and Manufacture Technique on Pellet Quality and Phytase Inclusion Levels on Broiler Performance

Tyler R Rigby

Experiments were conducted to determine the effects of different ingredients and manufacture techniques on the quality of pellets being produced as well as to examine how broiler performance will be affected by the dose of phytase product included in the diet. Chapter 3 explores the effects of a manipulation of either feed ingredient or manufacture technique (referred to as concern areas) on manufacture and pellet quality variables. Treatments included high or low mixer-added fat (2.5 vs. 0.5%), high or low distillers dried grains with solubles (DDGS) (8 vs. 2%), high or low dicalcium phosphate (1.63 vs. 0.31%), and high or low steam conditioning temperature (71 vs. 79°C). The high concern treatments for the mixer-added fat and dicalcium phosphate treatments demonstrated negative effects on pellet quality variables. However, the high concern treatments for DDGS and steam conditioning temperature did not produce similar negative effects. In chapter 4, different doses of phytase products were examined to determine an effective dosage level of each product on bird performance and tibia mineralization. Feed formulations were based on the phytase activity provided by the manufacturer and provided a calculated activity of 500, 1,500, and 6,000 FTU/kg for each phytase product. In addition, a positive and negative control diet based on non-phytate phosphorus level were formulated. Pellets were crumbled to a similar size and fed to 12 replicate pens of 10 straight-run Hubbard x Cobb broiler chicks for 21-d. Positive and negative control formulations produced relative differences in live weight gain, ending bird weight, and feed conversion ratio as expected. Main effects of dose and phytase product significantly affected live weight gain, ending bird weight, and feed conversion ratio. The granulated bacterial phytase was superior to the transgenic phytase corn product and the 6,000 FTU/kg dose was superior to the 500 and 1,500 FTU/kg doses.
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KEY

CHAPTER TWO

DDGS: distillers dried grains with solubles
IP6: myo-inositol 1,2,3,4,5,6-hexakis dihydrogen phosphate or phytate

CHAPTER THREE

DDGS: distillers dried grains with solubles
MAF: mixer-added fat
PDI: pellet durability index
MPDI: modified pellet durability index
PMEEU: pellet mill electrical energy usage
CEEU: conditioner electrical energy usage
DCP: dicalcium phosphate
MBM: meat and bone meal
NHPT: New Holmen Pellet Tester
ASAE: American Society of Agricultural Engineers

CHAPTER FOUR

FTU: phytase unit
d: day
P: phosphorus
IP6: myo-inositol 1,2,3,4,5,6-hexakis dihydrogen phosphate or phytate
TPC: transgenic phytase corn
GBP: granulated bacterial phytase
NHPT: New Holmen Pellet Tester
AOAC: Association of Analytical Chemists
LWG: live weight gain
FCR: feed conversion ratio
CHAPTER ONE
INTRODUCTION

The world’s population is expected to exceed 9 billion people by the year 2050, and if current methods are continued, the world will not be able to feed itself (Zeigler and Steensland, 2015). Therefore the agriculture industry is faced with the task of improving the methods by which food is produced so it will be better equipped to feed the increased number of people in the world. World poultry consumption has been estimated to increase from the number four animal protein consumed in 1964-1966 to the number two consumed animal protein in 2015; additionally, it is further estimated that by 2030, poultry will become the number one consumed animal protein in the world (Alexandratos et al, 2003).

Due to this continued increase in poultry consumption, the industry is faced with the task of producing poultry products more efficiently and for the least cost possible. Approximately 60-70% of the costs associated with broiler production are contributed by the feed (Behnke and Beyer, 2004). Feeding pelleted diets in the poultry industry is a widespread occurrence. This is largely due to the abundant amount of research proving the benefits that are associated with pelleted diets when compared to mash diets. As far back as the 1950s, improvements in bird growth were being observed when pellets were fed to broilers (Allred et al, 1957a; Allred et al, 1957b; Pepper et al, 1959; Jensen et al, 1962).

Further research has shown that higher quality pellets offer advantages to broilers. When high quality pellets are fed to broilers, performance (especially feed conversion ratios) can be improved (Moritz et al, 2002; Cutlip et al, 2008; Lilly et al, 2011; Wamsley and Moritz, 2014; Glover et al, 2016; Lemons et al, 2016). Improved feed conversion ratios can help decrease the costs faced by the producer, and therefore the consumers, since the birds are able to utilize more of the nutrients found in the feed while consuming less feed.
However, feeding high quality pellets is not the only way by which feed conversion ratios can be improved. An improvement in the feed efficiency (i.e. feed:gain ratio or feed conversion ratio) associated with a negative control can also be observed when phytase products are included in the diet (Tang et al, 2012). The use of a super-dose (an inclusion of phytase that is greater than 2,500 FTU/kg (Cowieson et al, 2011)) of phytase has been investigated to see how broiler performance is affected. Shirley and Edwards (2003) found that feeding a super-dose of phytase will further enhance the improvement in feed:gain ratios.

Therefore, a large amount of research has been conducted within the poultry industry focusing on how poultry production can be improved. Improvements can be accomplished through feed ingredients, manufacture techniques, pellet quality, or enzyme inclusions and can be measured through variables such as feed intake, live weigh gain, and feed conversion ratios. The following chapters examine how improvements can be made utilizing the aforementioned changes.

References


CHAPTER TWO
LITERATURE REVIEW

I. Pellet Quality

The poultry industry is constantly concerned with how to improve the way broilers are produced to ensure that the shortest period of time is utilized and the least amount of costs are incurred. Research focusing on these improvements has been conducted for many years to establish the best method by which this can be accomplished. As early as the 1950’s, it was found that feeding pelleted diets as opposed to mash diets will improve bird growth (Corzo et al., 2011; Jensen et al., 1962; Pepper et al., 1959; Allred et al., 1957a; Allred et al., 1957b). Allred et al (1957a, 1957b) suggested that a chemical change occurs within the feed during the pelleting process which leads to the advantages observed.

With pelleted feeds being fed almost exclusively in the poultry industry, there is great interest in how to better produce pellets. There are many advantages associated with pelleting feeds including increased animal performance, decreased feed wastage, reduced selective feeding, improved bulk density, better material handling characteristics, and the destruction of deleterious organisms within feed (Behnke, 1994). More recent research has found that there is also an advantage associated with the quality of the pellets being fed. Many recent publications found that feed conversion ratios will improve and feed intake will decrease when better quality pellets are fed when compared to a lower quality pellet (Glover et al., 2016; Lemons et al., 2016; Wamsley and Moritz, 2014; Lilly et al., 2011; Cutlip et al., 2008; Moritz et al, 2002).

Additionally, pellet quality can be affected by several factors, including diet formulation, ingredient particle size, mash conditioning temperature, feed rate, die speed, die specifications, etc. (Loar and Corzo, 2011; Behnke, 1994). Diet formulations, especially ingredient choices, are of great concern since each ingredient could have specific limitations that would need to be
overcome. One such limitation can occur when different corn varieties (i.e. normal dent, waxy, high-oil, waxy-high-oil (Zarate et al, 2004 as reported by Loar and Corzo, 2011)) are utilized in the diet; another feed ingredient which could affect pellet quality is corn distillers dried grains with solubles (Loar and Corzo, 2011). Pellet quality can be improved if pellet binders, such as bentonite and lignosulphonates, are included in the diets (Loar and Corzo, 2011; Thomas et al, 1998).

II. Corn Distillers Dried Grains with Solubles (DDGS)

In recent years more corn is being converted into ethanol. This is a concern for feed manufacturers, and therefore producers, because the price of corn is being driven up. With the costs of corn rising, nutritionists and feed manufacturers are focusing on ways to better lower feed costs by incorporating lower corn inclusions. One method they can utilize is having a portion of the corn be replaced with corn distillers dried grains with solubles (DDGS). However, the nutrient profile of DDGS products can vary greatly (Behnke, 2007; Belyea et al, 2004; Spiehs et al, 2002).

Historically, DDGS were the by-product of the alcohol industry (Behnke, 2007). However, with the realization that burning ethanol is much cleaner, more DDGS are being produced through this industry than the alcohol industry (Lumpkins et al, 2004). The production of DDGS in a conventional dry grind process consists of “corn being ground using a hammermill. The ground corn is then mixed with water to form a slurry. This slurry is then cooked at about 160°C using pressurized steam which breaks down the crystalline structure of starch. Alpha amylase is then added to break down the starch polymers to dextrin in order to form a mash. This mash is then held briefly at a high temp (about 70°C) before being cooled to 32°C and transferred to fermentation tanks. Glucoamylase and yeast are added to the
fermentation tank; the glucoamylase takes all dextrins and breaks them down to glucose and maltose while the yeast ferments the glucose and maltose into ethanol. What is left after ethanol is removed is dried and can be sold as DDGS (Singh et al, 2007).”

The use of DDGS in animal diets has been widely used in the dairy and beef sectors of animal production; however, the use of DDGS in poultry and swine diets is relatively new (Shurson and Noll, 2005). It has been found that DDGS can be included in poultry diets at levels of 5% or lower without having any negative effects on bird performance (Lumpkins et al, 2004). However, more recent research has investigated the possibility of differing inclusions of DDGS based on the growth phase of the bird. Swiatkiewicz and Koreleski (2008) reported that a high quality DDGS product can be safely fed to broiler chickens and turkeys who are in the starter growth phase at inclusion levels of 5-8%; however, DDGS inclusions of 10-15% can be included in diets for broilers and turkeys who are in the grower-finisher growth phase as well as laying hen diets.

III. Mixer-Added Fat

Fat is utilized in poultry diets as a way to increase the amount of energy being provided to the bird (Schaible, 1970b). However, there is a great concern that is associated with adding too much fat to the diets prior to pelleting, this is likely a result of the hydrophobic nature of fat interfering with the denaturation of the natural bonds in the feed ingredients used (Loar and Corzo, 2011). When large amounts of fat are added at the mixer, there is a decrease in the pellet quality normally observed (Loar et al, 2014). As a result of the excess fat, the feed may not spend enough time in the die which could lead to the starch gelatinization and protein gelation not occurring resulting in a pellet of poor quality. The excess fat added at the mixer can also effected starch gelatinization since the excess fat will coat the starch molecule making it more
difficult for the hydrogens released from hydrogen bonds in amylose to leave the starch molecule and reform new hydrogen bonds with similarly released hydrogens resulting in a sticky substance which aids in forming higher quality pellets (Thomas et al., 1998). When only a portion of fat is added at the mixer, the remaining fat inclusion is added to the pellets as a post-pellet application. Since pellet quality is affected by the mixer-added fat inclusion, there is an indirect effect of fat inclusions on bird performance (Glover et al., 2016; Lemons et al., 2016; Wamsley and Moritz, 2014; Lilly et al., 2011; Cutlip et al., 2008; Moritz et al., 2002).

In addition to influencing the quality of pellets produced, mixer-added fat levels have other effects on the pellet production process. When higher levels of fat are added at the mixer, the pellet mill electrical energy usage will be decreased when compared to lower levels of mixer-added fat (Loar et al., 2014; Gehring et al., 2011).

IV. Steam Conditioning Temperature

There are many benefits that can be associated with conditioning feeds prior to pelleting. Bayley et al (1968b) suggested that utilizing steam in the pelleting process will increase the availability of phosphorus found in the plant portion of corn-soybean meal based diets. In 1968(a), Bayley and coauthors reported that utilizing a higher conditioning temperature (about 90°C) will reduce bird performance when compared to birds fed pellets that were conditioned at 70°C. However, more recent research has shown that higher conditioning temperatures will produce a higher quality pellet (Loar et al., 2014; Cutlip et al., 2008), thus enhancing bird performance (Glover et al., 2016; Lemons et al., 2016; Wamsley and Moritz, 2014; Lilly et al., 2011; Cutlip et al., 2008; Moritz et al., 2002). The higher quality pellets were likely produced due to an increase in lubrication at the die as well as an increase in adhesion of the feed particles (Cutlip et al., 2008). Pellet quality may also be affected by the steam conditioning temperature
through starch gelatinization. Starch gelatinization relies on heat to destroy the naturally occurring bonds in starch so that a reordering of the molecules can occur (Schwartz and Zelinskie, 1978 as reported by Thomas et al, 1998). In addition to the pellet quality effects, the steam conditioning temperature utilized can also affect other feed manufacture variables. The energy used by the pellet mill will decrease as the steam conditioning temperature utilized increases due to the extra steam needed to reach the higher temperature having a lubricating effect on the pellet die which reduced the amount of energy needed to extrude the mash diet (Loar et al, 2014).

V. Rock Phosphate

The production of rock phosphates has been well established, with patents being recorded for the methods by which to produce various forms of rock phosphate as far back as 1917 (Ogburn et al, 1942; Curtis, 1936; Glaeser, 1917). However, it wasn’t until 1955 that the form most utilized in diets today (dicalcium phosphate) was produced at a feed grade level (Miller, 1955). Further progress has been made with the acquisitions of patents for a newer method of dicalcium phosphate (Fallin, 1958) as well as for the production of a granulated dicalcium phosphate (Baron, 1959).

With large amounts of phosphorus being trapped in plant materials which are unusable by poultry and swine, nutritionists have had to find another source by which to add phosphorus to the diets (Angel et al, 2002; Ravindran et al, 1994). As a result of this, the addition of rock phosphates to diets has become a common occurrence. While it is almost a standard procedure to include dicalcium phosphate in the diet formulation, there is very little published research examining the effect of dicalcium phosphate on pellet quality. Wamsley and coauthors (2012) found that variations in the rock phosphate source, as well as the particle size of the rock
phosphate source, utilized have varying effects on pellet quality. Tricalcium phosphate included in diets, either at a regular or fine particle size, has shown to increase production rate when compared to a diet containing dicalcium phosphate (Behnke, 1981 as reported by Loar and Corzo, 2011). Wamsley and coauthors (2012) speculated that tricalcium phosphate effects production rate by increasing the die scouring that occurs. Additionally they believed that this increase is due to the density, particle size, and shape of the tricalcium phosphate when compared to both diacalcium and monocalcium phosphate (Wamsley et al, 2012).

VI. Meat and Bone Meal

Meat and bone meals are a by-product that are produced by the rendering industry (Karakas et al, 2001). Including meat and bone meal in poultry diets can offer advantages as well as possible drawbacks. One benefit of adding meat and bone meal to broiler diets would be a decrease in the amount of rock phosphate being included. This is possible because meat and bone meal contains highly available phosphorus, thus reducing the amount of phosphorus that needs to be included through rock phosphates. Another benefit of including more meat and bone meal is the reduced price associated with it when compared to dicalcium phosphate inclusions; the cost of a 50 pound bag of meat and bone meal is approximately $9.13 (USDA-MO Dept. of Ag. Market News, 2016) while the cost of a 50 pound bag of dicalcium phosphate is approximately $25.99 (Mills Fleet Farm). However, as meat and bone meals are considered for inclusions in animal diets, the diet formulator must be consciences of the possible variability of the amino acid composition in the product utilized (Hendriks et al, 2002; Karakas et al, 2001; Wang and Parsons, 1998; Parsons et al, 1997). Drewyor and Waldroup (2000) demonstrated that adding a meat and bone meal that was either high or low ash will not have a significant effect on broiler body weight and feed utilization when it is incorporated into a balanced nutritional diet. In
addition to amino acid concerns, meat and bone meal also raises concerns with metabolizable energy since the majority of the research conducted reports metabolizable energy in terms of apparent or true which is normally determined utilizing a cecetomized cockerel who has a different physiological state than a broiler chicken (Karakas et al, 2001).

VII. Moisture Effects

It has been established that by incorporating an increase in mash moisture content by 3% will lead to a 2.3% decrease in the use of energy while increasing the durability of the pellets produced by 10% (Moritz et al, 2001). Thomas and coauthors in 1997, recounted that the presence of water will facilitate gelatinization, denaturation, and solubilization within feed ingredients similar to what is observed when steam conditioning is done. As moisture is added to industry recommended and low density diets, an associated improvement of production rate and decrease of pellet mill electrical energy usage are observed (Moritz et al, 2003). Moritz et al (2001) reported that the addition of moisture at the mixer resulted in higher pellet durability and starch gelatinization; they attributed the higher starch gelatinization to the presence of water 1) being a prerequisite for the gelatinization to occur and 2) limiting the full gelatinization of the starch contained within the diets. Additionally, it was suggested that by adding moisture at the mixer, a portion of the problems encountered when high levels of fat are added at the mixer can be overcome (Moritz et al, 2002). However, as moisture levels increase, a greater concern arises in terms of mold risks; with this in mind, commercial mold inhibitors are being added to feeds (Hott et al, 2008). Hott and coauthors (2008) found that after one day post pelleting, there was no significant difference in moisture between treatments regardless of the amount of added moisture prior to pelleting.

VIII. Phytate Phosphorus and Phytases
The phosphorus requirement of chickens, especially with respect to how to best provide the required amount in the feed, has been an ongoing concern for the poultry industry. O’Rourke and associates addressed the requirements of the chicken at the Tenth World’s Poultry Congress in 1954. Additionally, the phosphorus content of different products utilized varies greatly (Schaible, 1970a).

With the majority of poultry diets being plant based, the majority of the phosphorus contained in the ingredients is largely indigestible to monogatsric animals since it is trapped as phytate (myo-inositol 1,2,3,4,5,6-hexakis dihydrogen phosphate or IP6) (Cowieson et al, 2011; Angel et al, 2002; Ravindran et al, 1994; O’Rourke et al, 1954). When IP6 chelates with Ca, Mg, and K to form a complex salt, IP6 can be referred to as phytin (Angel et al, 2002). It has been reported that chicks and turkeys between the ages of 8 and 20 weeks cannot utilize any large amount of phytin (Schaible, 1970a).

More recent research has focused on phytase products. Phytases (myo-inositol hexaphosphate hydrolases) are able to hydrolyze bonds found in IP6 (Angel et al, 2002). There are two recognized classifications into which phytase products can be divided, either a 3-phytase or a 6-phytase; these classifications are distinguished by the location on the inositol ring where the phytase begins the dephosphorylation process (Angel et al, 2002). When phytase hydrolyzes the IP6 bonds, the IP6 is broken down into much smaller molecules (Angel et al, 2002). Since the particles moving through the bird’s gastro-intestinal tract are smaller it can be speculated that there will be a reduction in the irritation observed which will lead to a decrease in the amount of energy that the bird needs to expend towards an immune response.

When varying doses of phytase products are included in the diets of broiler chickens, a faster growth can be observed when compared to a positive control diet (Tang et al, 2012).
Additionally, the authors found that feed intake levels were increased for the birds who were fed the phytase diets as opposed to the birds fed the positive control diet; however, the feed conversion ratios did not produce a significant difference. Cowieson et al (2006) found that body weight gain and feed conversion ratios for birds fed supplemented negative control treatments resulted in values that were similar to those obtained from the birds fed the positive control treatment.

Further research has been conducted to examine the use of super-doses of phytase in poultry diets. A super-dose is defined as a phytase inclusion that is greater than 2,500 FTU/kg of either an \textit{Aspergillus niger} or \textit{Escherichia coli} derived phytase (Cowieson et al, 2011). Shirley and Edwards (2003) first investigated how super-doses will effect broiler performance; they found that broilers who were fed super-doses will have improved bird weight gain, feed intake, and feed:gain ratios. In addition to liberating trapped phosphorus from IP6, super-doses of phytase can increase inositol concentrations in the gizzard, almost completely hydrolyze IP6, and alleviate some of the antinutrient effects associated with phytate presence in the diet (Walk et al, 2014; Walk et al, 2013).

\section*{IX. Transgenic Phytase Products}

The use of transgenic phytase products is relatively new, with its first introduction being in 1993 by Pen and coauthors who investigated the effects of using a tobacco seed harboring an \textit{Aspergillus niger} derived phytase enzyme. They found that the inclusion of the transgenic tobacco seeds in broiler diets resulted in an improved utilization of phosphorus and weight gain when non-phytate phosphorus levels were low as well as body weights that were similar to diets that contained commercial \textit{A. niger} phytase products. While this research is important, it is not exactly applicable to the poultry industry since tobacco seeds are not a traditional feed ingredient
(Foltz, 2016). More applicable work has been conducted utilizing transgenic corn and transgenic soybeans in broiler (Nyannor et al, 2009; Nyannor and Adeola, 2008; Denbow et al, 1998), roosters (Gao et al, 2012), and laying hen (Gao et al, 2013) diets. Nyannor and Adeola (2008) reported that the utilization of a transgenic phytase corn increased weight gain when compared to both a positive and negative control as well as to a commercial phytase product. Denbow et al (1998) found similar results as Nyannor and Adeola (2008) when examining transgenic soybeans.

X. Environmental Effects of Phosphorus

A major concern for environmentalists revolve around eutrophication of waterways. One of the big causes of eutrophication is an excess in nutrients in the water, especially phosphorus (Tang et al, 2012; Correll, 1999). Historically, the environmental effects of phosphorus were not always well known; however, as more research has been conducted, scientists have a greater understanding of the effects of phosphorus. With this in mind, farmers are being cautioned of the use of fertilizer on their fields since the phosphorus being stored in the soil or freshly applied to the field can be leached into the water system (Hart et al, 2004; Angel et al, 2002).

The effects of phosphorus on the environment is of great concern to the poultry industry since poultry excreta could potentially contain high levels of phosphorus should a phytase product not be included in the diet (Tang et al, 2012). Therefore, when the fertilizer derived from poultry manure is applied to the fields, there is a greater risk of environmental consequences (Angel et al, 2002).

XI. References


Mills Fleet Farm. 2016. Sprout 18.5% Dicalcium Phosphate – 50 lb.

http://www.fleetfarm.com/detail/sprout-18-5-dicalcium-phosphate-50-lb-
/000000201642


CHAPTER THREE
Effects of modifying diet and feed manufacture concern areas that are notorious for decreasing pellet quality


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Primary Audience: Nutritionists, Feed Mill Managers, Researchers

SUMMARY

The production of high quality pellets has often been described as more art than science due to the multitude of variables that affect pellet quality. The objectives of the current study were to examine the feed manufacture and pellet quality effects of four different feed milling concern areas. Each area of concern was evaluated using either a condition of high concern or a corresponding condition of low concern. Treatments included high or low mixer-added fat (2.5 vs. 0.5%), high or low distillers dried grains with solubles (DDGS) (8 vs. 2%), high or low dicalcium phosphate (1.63 vs. 0.31%), and high or low steam conditioning temperature (71 vs. 79°C). All diets were formulated to similar broiler starter requirements based on commercial nutrient values. A randomized complete block design using a 4 x 2 factorial arrangement was implemented with three replications per treatment and 136 kg allotments of feed as the experimental unit. Contrasts were performed to better understand main effect interactions. High mixer-added fat and low dicalcium phosphate decreased pellet durability, percent pellets, and pellet size (P<0.05). Conditions of high concern for DDGS and steam conditioning did not produce similar negative effects (P>0.05). Pellet mill energy usage was only affected due to mixer-added fat (P<0.05). Additional diet variables such as corn moisture content and nitrogen free extract of DDGS likely confounded treatment effects. A greater appreciation of variable interactive effects benefits pellet mill operators, nutritionists, and pelleting aid vendors to better circumvent hurdles encountered during the pelleting process.

Key words: pellets, fat, DDGS, dicalcium phosphate, steam conditioning
DESCRIPTION OF PROBLEM

The production of high quality pellets is complex due to the multitude of variables that affect feed manufacture and pellet quality. Feeding high quality pellets has consistently been demonstrated to improve poultry performance [1-7]. Feed and feed manufacture costs, which comprise the majority of costs associated with poultry production [8], warrant emphasis on proper pellet production [9].

Feed manufacture research is sparse compared to other fields of poultry science. However, the feed manufacture industry abounds with anecdotal information from the experience of feed mill managers and pellet mill operators. High corn distillers dried grains with solubles (DDGS) and mixer-added fat (MAF) inclusions are notorious for complicating feed manufacture and decreasing pellet quality. Similarly, low conditioning temperatures and rock phosphorus inclusions are believed to be detrimental to the pelleting process.

Past research has shown that pellet quality suffers when increasing inclusions of DDGS [10-11], decreased steam conditioning temperatures [6, 12-14], and high amounts of MAF [14, 16-17] are factored into the diet formulation and/or manufacturing process. Additionally, it has been found that pellet quality will vary based on the rock phosphate source used [18].

Behnke [11] states that pellet quality will suffer, along with pellet throughput, with inclusions of DDGS greater than 5-7%. Additionally, it has been found that pellet quality in terms of the pellet durability index (PDI) and modified pellet durability index (MPDI) decrease as DDGS inclusions increase [10]. Additionally, Loar, et al [10] found that high inclusions of DDGS will result in a higher pellet mill electrical energy usage (PMEEU) but a lower conditioner electrical energy usage (CEEU). When looking at the steam conditioning temperature effects, Loar and coauthors [14] reported that increasing the conditioning
temperature from 74°C to 85 or 96°C will result in a decrease in PMEEU. Additionally, it was reported that as the steam conditioning temperature increases so does the pellet quality as measured by PDI and MPDI [6, 14]. Gehring, et al [16] found that higher inclusions of MAF will result in a decreased PDI and MPDI. Other research has shown that PMEEU will decrease as MAF increases [17]. Wamsley, et al [18] reported that the use of tricalcium and dicalcium phosphates will cause increases in PDI and MPDI, while decreasing PMEEU.

The objectives of the current study were to examine the feed manufacture and pellet quality effects of four different milling concern areas. Each area of concern was evaluated using either a condition of high concern or a corresponding condition of low concern. Treatments included high or low diets for MAF (2.5 vs. 0.5%), DDGS (8 vs. 2%), rock phosphate (dicalcium phosphate (DCP)) (0.31 vs. 1.63%), and steam conditioning temperature (71 vs. 79°C).

MATERIALS AND METHODS

Treatment Structure and Diet Preparation

Experimental treatments were structured as an area of concern (4) x level of concern (2) factorial arrangement within a randomized complete block design. All diets were formulated to similar calculated nutrient values based on commercial (for energy and available phosphorus content) [19] and research (for amino acid inclusions) recommendations [20] (Table 1). Feed manufacture was conducted at the West Virginia University pilot feed mill. The feed was batched and mixed using a one-ton vertical screw Easy Automation Inc. mixer [21]. Master batches of feed were mixed and split so that all basal ingredients would be similar among treatments. The DDGS levels were balanced by manipulating corn and soybean meal inclusions. The rock phosphate levels were balanced in part with porcine meat and bone meal (MBM). The MAF and steam conditioning temperature concern area diets utilized similar diet formulations.
136 kg aliquot of feed represented the experimental unit to be conditioned and pelleted. Treatments that did not emphasize MAF utilized 1.5% soybean oil addition at the mixer with the remaining fat added post pelleting. Each area of concern was divided into a high and low level of concern. The following outline may best depict treatment structure.

**Corn DDGS**
- High (8%)
- Low (2%)

**Rock phosphate (DCP)**
- High (0.31%)
- Low (1.63%)

**MAF**
- High (2.5%)
- Low (0.5%)

**Conditioning temperature**
- High (71°C)
- Low (79°C)

*Feed Manufacture*

Experimental treatments were steam conditioned using a 1.3 x 0.31 m short-term (10 s) California Pellet Mill conditioner at 79°C (except for the treatment testing the conditioning temperature which was conditioned at 71°C). The feed was then extruded through a 5 x 38 mm pellet die driven by a 40-horsepower California Pellet Mill [22].

The night before pelleting, one of the 136-kg aliquots for each of the eight diets was placed into an environmentally controlled room where temperature was held constant at 95°F in
an attempt to pre-warm the feed. This was done in an attempt to help combat the low ambient temperatures experienced on pelleting days (31-33°F, 27-41°F, and 51-57°F for days 1, 2, and 3 respectively). A 90.7-kg amount of a standard poultry diet was used each day prior to pelleting experimental diets to warm-up the pellet die and minimize the time necessary to obtain goal conditioning temperature for experimental treatments.

Feed manufacture measured variables included: production rate, CEEU, and PMEEU (Table 2). Post pelleting, the remaining fat as dictated by the diet formulation (Table 1) was added to pellets within the vertical mixer for a two minute mix time. Pellet quality tests were conducted 24 hours post-pelleting/mixing. These tests included pellet survivability, pellet/crumble/fine percentage, and pellet particle size (Table 3). Pellet survivability was tested by utilizing a New Holmen Pellet Tester (NHPT) [23]. Pellet, crumble, and fine percentages were calculated by sieving a sample of approximately 23-kg of feed per treatment through an American Society of Agricultural Engineers (ASAE) #5 and #14 sieve [24].

A 100 gram sample was collected from each treatment post-pelleting for each day of feed manufacture. At the end of the three days, the three samples were compiled and blended. From this blended sample a representative 100 gram sample was collected and sent to a commercial laboratory for proximate analysis (Table 1).

**Statistical Analysis**

Feed manufacture and pellet quality data were analyzed using a 4 x 2 factorial arrangement 2-way ANOVA in a randomized complete block design. Day of feed manufacture was used for the blocking criteria. Main effects and interactions were considered and if interactions were significant then Fisher’s LSD multiple comparison test was used to separate means. Linear contrasts were also performed to help further explain interactions as well as
differences between high and low concerns within each concern area. Significance was determined at $P < 0.05$.

**RESULTS AND DISCUSSION**

Main effects and interaction data are presented in Table 2, and contrasts are presented in Table 3. In addition, Table 4 contains analyzed values for selected dietary ingredients that may help to explain treatment effects.

The only feed manufacture variable to demonstrate a concern area x level of concern interaction was hot pellet temperature ($P = 0.0057$, Table 2). These data with support from the contrast data (Table 3), show that only the high and low concern conditioning temperature influenced hot pellet temperature. This effect was intuitive, i.e. 79°C conditioning temperature increased hot pellet temperature compared to 71°C conditioning temperature. Hot pellet temperature may also indicate frictional heat generation within the pellet die. However, treatment effects on frictional heat generation were likely not enough to influence hot pellet temperature. Moisture level of corn used in the diet formulation (Table 4) may have increased lubrication and partially masked beneficial or detrimental effects of treatments on pellet die frictional heat and hot pellet temperature as well as the additional feed manufacture variables measured. Low ambient temperatures during feed manufacture and use of a high volume of steam to reach target conditioning temperatures may have had a similar influence. However, contrasts demonstrated that high levels of mixer-added fat decreased PMEEU ($P = 0.0099$, Table 3).

Multiple pellet quality variables were affected by a concern area x level of concern interaction ($P < 0.05$, Table 2). In general, the rock phosphorus and MAF treatments demonstrated decreased pellet quality at the high level of concern and DDGS and steam conditioning temperature were unaffected by level of concern (Table 3). The inconsistencies of
concern area across levels of concern may also be partially explained by dietary ingredient profiles (Table 4). The specific concern areas are discussed separately.

**DDGS**

Contrasts demonstrated that low and high concern levels for DDGS did not affect feed manufacture or pellet quality variables (P < 0.05, Table 3). These data contradict anecdotal information as well as Loar, et al [10] who found that higher inclusions of DDGS produced a lower PDI and MPDI while producing a higher percentage of fines. Wamsley, et al [25] also found that for starter diets, pellet quality was not significantly changed as DDGS inclusion increased from 0 to 4%. Perhaps variations on how DDGS affect feed manufacture and pellet quality are associated with variations in nutrient profile of DDGS due to ethanol processing. Spiehs, et al [26] examined the nutritional composition of DDGS products that were produced in Minnesota and South Dakota and found a great variation in the proximate analysis results; additionally, Behnke reported on the high variation in DDGS products available [11]. In the current study, the DDGS utilized contained 37.5% nitrogen free extract that could be comprised of starch with potential to partially gelatinize during the pelleting process. Starch gelatinization is a key reaction for pellet formation of corn and soybean based diets [2]. If DDGS starch is available for gelatinization then it could offset negative effects of DDGS oil and fiber, thus minimizing detrimental effects on pellet quality.

**Rock Phosphate (DCP)**

Pellet survivability (P = 0.0027), percent pellets produced (P = 0.0080), percent crumbles produced (P = 0.0016), and pellet particle size (P = 0.0004) all increased with higher inclusions of DCP (Table 3). The benefits of DCP relative to MBM may be similar to those noted for tricalcium phosphate compared to DCP in terms of pellet die scouring [18]. Wamsley, et al [18]
found that inclusions of DCP increased pellet quality when compared to inclusions of tricalcium phosphate in the experiment 1, but a similar pellet quality increase was not observed in experiment 2. However, no benefit was observed in terms of feed manufacture variables (P > 0.05). The lack of difference in feed manufacture of the current study may be attributed to corn moisture and low ambient temperatures that would necessitate a high volume of steam to meet goal conditioning temperatures. These variables would increase conditioned mash moisture and could confound effects of die scouring on throughput and electrical energy use. In addition, the porcine MBM used in the study may have had much of the protein denatured thus making it unavailable for protein gelation during the pelleting process since denaturation is an irreversible process. The authors speculate that had protein denaturation occurred during the production of the meat and bone meal product, the proteins may have been denatured too far in advance for the gelation to have occurred during the pelleting process. Thomas et al (1998) [27] reported that there are no reports concerning the effects of feed manufacture on protein denaturation or the effect it will have on pellet quality; additionally, they cite sources which emphasize the lack of knowledge we have concerning how proteins will react when in conjunction with other non-protein particles (i.e. a mash diet). Protein gelation is another key reaction for pellet formation [28-29].

**Steam Conditioning Temperature**

High and low concern treatments showed that there was no effect of steam conditioning temperature on pellet quality variables (P > 0.05, Table 3). Additionally, PMEEU, CEEU, and production rate all demonstrated no effect (P > 0.05) while hot pellet temperature (P < 0.05) decreased as the concern level increased (Table 3). These results are in opposition with Loar, et al [14] who found that higher steam conditioning temperatures will reduced the PMREE while
increasing PDI and MPDI. Cutlip and coauthors [6] also found that PDI and MPDI increased and total fines produced decreased as the steam conditioning temperature increased. The authors speculate that the results observed could be due to the semi-wet corn [30] utilized in the study raising the moisture content of the feed to a level at which there would be no positive benefit of adding additional moisture via steam.

**MAF**

Pellet survivability (P = 0.0059) along with percent pellets (P = 0.0012), percent crumbles (P = 0.0012), and percent fines produced (P = 0.0029) all increased as higher inclusions of fat at the mixer were utilized (Table 3). Corey and coauthors [17] reported similar results for pellet quality, showing that PDI and MPDI both decreased as MAF increased from 1 to 3%. This decrease is likely due to the higher MAF coating the starch molecule making it more difficult for the hydrogens released from hydrogen bonds in amylose to leave the starch molecule and reform new hydrogen bonds during starch gelatinization [31]. However, PMEEU (P = 0.0099, Table 3) decreased as more MAF was used. Gehring, et al [16] found similar decreases in PMEEU as MAF increased from 1 to 4%. This decrease in PMEEU is likely due to the fat coating the pellet die and allowing the conditioned mash to more easily move through the pellet die resulting in less energy having to be expended at the pellet mill. The pellet particle size, production rate, CEEU, and hot pellet temperature variables all returned non-significant (P > 0.05, Table 3).

In conclusion, we can see that the data obtained from the current study depicted that what we believe will happen based on either anecdotal information obtained from industry professionals as well as on what past research has shown in fact may not occur due to comprehensive interactions between variables. The results obtained during this study could have
been affected by several different confounding variables including the ambient temperatures during the production of feed and the high moisture content in the corn used for the study. The low ambient temperatures at the time of feed manufacture necessitated a larger amount of steam to be added to the feed in order for the target conditioning temperature to be reached. The semi-wet corn that was utilized in the diet formulations likely affected the moisture content of the feed prior to conditioning, resulting in the higher levels of steam additions being too much to have any added benefit during pelleting. Additionally, the higher moisture content of the unconditioned mash due to the semi-wet corn may have prevented expected treatment results from being observed. Past research has shown that as moisture content in the unconditioned mash increases, a positive result occurred in the quality of the pellets produced [2-3, 32].

CONCLUSIONS AND APPLICATIONS

1. The effects of corn DDGS on feed manufacture and pellet quality variables may not exclusively be negative due to confounding interactions of variables.

2. Despite any confounding variable interactions, the effects of higher DCP inclusions presented a positive result on both feed manufacture and pellet quality variables; however, some results could have been influenced by the addition of MBM in the diets.

3. When confounding interactions of variables occur, low steam conditioning temperatures may not have an exclusively negative effect on feed manufacture and pellet quality variables.

4. In conjunction with confounding variable interactions, MAF of a high level of concern affected pellet quality variables in a negative manner and decreased electrical energy use of the pellet mill.

REFERENCES AND NOTES


[19] Metabolizable Energy and Available Phosphorus were based on Agristat values as suggested by M. Donohue. 2013. The Challenges in Feeding Broilers in Times of High and Volatile Feed Ingredient Costs: How to Cover the Costs?. 2013 Mid-Atlantic Nutrition Conference proceedings. Calcium values were adjusted to a 2.1:1 of Calcium to Available phosphorus.


[23] Pellet quality was assessed one day following production using the New Holmen Portable Pellet Durability Tester, Lignotech USA, INC., Rothschild, WI. 100 g of pellets were sifted through No. 5 American Society for Testing and Materials (ASTM) screen and placed in holding chamber, blown for 30 s by a jet of air, then weighed, giving a direct read of pellet durability. Fine is removed during the blowing process.
[24] Pellets were defined as the feed that remained on the ASAE #6 sieve. Crumbles were defined as the feed that remained on the ASAE #14 sieve. Fines were defined as the feed that passes through the ASAE #14 sieve.


[30] Semi-wet corn is defined as corn with a moisture content greater than 15%. Corn may be partially dried in the field, and not further dried post-harvest upon delivery to a buyer. These semi-wet corn purchases may be blended with more dry corn allotments or expected to further dry during storage. Johnson, B. 2016. Kalmbach Feeds. Personal communication.

Table 1. Diet formulations and nutrient parameters.

<table>
<thead>
<tr>
<th>Ingredients</th>
<th>Low DDGS</th>
<th>High DDGS</th>
<th>Low Fat</th>
<th>High Fat</th>
<th>High Dical Low MBM (%)</th>
<th>High Dical High MBM (%)</th>
<th>Standard Conditioning Temp (79°C)</th>
<th>Low Conditioning Temp (71°C)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Corn</td>
<td>55.37</td>
<td>50.34</td>
<td>54.84</td>
<td>54.84</td>
<td>56.81</td>
<td>58.32</td>
<td>54.84</td>
<td>54.84</td>
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<tr>
<td>Soybean meal (48%)</td>
<td>33.80</td>
<td>32.18</td>
<td>36.48</td>
<td>36.48</td>
<td>33.47</td>
<td>28.72</td>
<td>36.48</td>
<td>36.48</td>
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<td>Corn DDGS</td>
<td>2.89</td>
<td>8.00</td>
<td>0.00</td>
<td>0.00</td>
<td>0.00</td>
<td>0.00</td>
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<td>0.00</td>
</tr>
<tr>
<td>Meat and Bone meal</td>
<td>0.00</td>
<td>0.00</td>
<td>0.00</td>
<td>0.00</td>
<td>2.00</td>
<td>8.00</td>
<td>0.00</td>
<td>0.00</td>
</tr>
<tr>
<td>Soybean oil</td>
<td>3.69 (1.5% at the mixer)</td>
<td>4.47 (1.5% at the mixer)</td>
<td>3.62 (0.50% at the mixer)</td>
<td>3.62 (2.50% at the mixer)</td>
<td>3.30 (1.5% at the mixer)</td>
<td>2.74 (1.5% at the mixer)</td>
<td>3.62 (1.5% at the mixer)</td>
<td>3.62 (1.5% at the mixer)</td>
</tr>
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<td>Dicalcium phosphate</td>
<td>2.07</td>
<td>2.05</td>
<td>2.07</td>
<td>2.07</td>
<td>1.63</td>
<td>0.31</td>
<td>2.07</td>
<td>2.07</td>
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<tr>
<td>Limestone</td>
<td>1.48</td>
<td>1.48</td>
<td>1.48</td>
<td>1.48</td>
<td>1.23</td>
<td>0.49</td>
<td>1.48</td>
<td>1.48</td>
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<tr>
<td>DL – methionine</td>
<td>0.50</td>
<td>0.44</td>
<td>0.50</td>
<td>0.50</td>
<td>0.50</td>
<td>0.46</td>
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<td>0.15</td>
<td>0.15</td>
<td>0.15</td>
<td>0.15</td>
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<tr>
<td>Vitamin premix</td>
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<td>0.25</td>
<td>0.25</td>
<td>0.25</td>
<td>0.25</td>
<td>0.25</td>
<td>0.25</td>
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<tr>
<td>Lysine HCl</td>
<td>0.24</td>
<td>0.27</td>
<td>0.17</td>
<td>0.17</td>
<td>0.22</td>
<td>0.24</td>
<td>0.17</td>
<td>0.17</td>
</tr>
<tr>
<td>Salt</td>
<td>0.27</td>
<td>0.21</td>
<td>0.29</td>
<td>0.29</td>
<td>0.26</td>
<td>0.15</td>
<td>0.29</td>
<td>0.29</td>
</tr>
<tr>
<td>Threonine</td>
<td>0.17</td>
<td>0.17</td>
<td>0.14</td>
<td>0.14</td>
<td>0.17</td>
<td>0.18</td>
<td>0.14</td>
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Calculated Nutrients:

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<tr>
<th>Low DDGS</th>
<th>High DDGS</th>
<th>Low Fat</th>
<th>High Fat</th>
<th>High Dical Low MBM (%)</th>
<th>High Dical High MBM (%)</th>
<th>Standard Conditioning Temp (79°C)</th>
<th>Low Conditioning Temp (71°C)</th>
</tr>
</thead>
<tbody>
<tr>
<td>ME (kcal/lb)</td>
<td>1361</td>
<td>1361</td>
<td>1361</td>
<td>1361</td>
<td>1361</td>
<td>1361</td>
<td>1361</td>
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<tr>
<td>Lysine (%)</td>
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<td>1.20</td>
<td>1.20</td>
<td>1.20</td>
<td>1.20</td>
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<tr>
<td>Methionine + Cysteine (%)</td>
<td>1.13</td>
<td>1.08</td>
<td>1.14</td>
<td>1.14</td>
<td>1.13</td>
<td>1.09</td>
<td>1.14</td>
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<tr>
<td>Threonine (%)</td>
<td>0.84</td>
<td>0.84</td>
<td>0.84</td>
<td>0.84</td>
<td>0.84</td>
<td>0.84</td>
<td>0.84</td>
</tr>
<tr>
<td>Tryptophan (%)</td>
<td>0.22</td>
<td>0.21</td>
<td>0.23</td>
<td>0.23</td>
<td>0.22</td>
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<td>0.23</td>
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<tr>
<td>Calcium (%)</td>
<td>1.08</td>
<td>1.08</td>
<td>1.08</td>
<td>1.08</td>
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<td>1.08</td>
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<tr>
<td>Available P (%)</td>
<td>0.51</td>
<td>0.51</td>
<td>0.51</td>
<td>0.51</td>
<td>0.51</td>
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<tr>
<td>Sodium (%)</td>
<td>0.17</td>
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<td>0.17</td>
<td>0.17</td>
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Nutrient Analysis:

<table>
<thead>
<tr>
<th>Low DDGS</th>
<th>High DDGS</th>
<th>Low Fat</th>
<th>High Fat</th>
<th>High Dical Low MBM</th>
<th>High Dical High MBM</th>
<th>Standard Conditioning Temp (79°C)</th>
<th>Low Conditioning Temp (71°C)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Moisture Content (%)</td>
<td>14.9</td>
<td>14.7</td>
<td>15.3</td>
<td>15.2</td>
<td>15.1</td>
<td>15.0</td>
<td>15.1</td>
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<tr>
<td>Protein Content (%)</td>
<td>21.6</td>
<td>22.0</td>
<td>21.8</td>
<td>21.8</td>
<td>22.1</td>
<td>21.7</td>
<td>22.1</td>
</tr>
<tr>
<td>Fat Content (%)</td>
<td>6.95</td>
<td>7.08</td>
<td>6.57</td>
<td>6.79</td>
<td>6.16</td>
<td>6.50</td>
<td>6.37</td>
</tr>
<tr>
<td>Crude Fiber Content (%)</td>
<td>2.03</td>
<td>3.09</td>
<td>2.62</td>
<td>2.54</td>
<td>2.16</td>
<td>1.77</td>
<td>3.15</td>
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<tr>
<td>Ash Content (%)</td>
<td>5.33</td>
<td>5.48</td>
<td>5.57</td>
<td>5.43</td>
<td>5.35</td>
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<td>5.62</td>
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<tr>
<td>Phytic acid Ion Exchange (%)</td>
<td>0.868</td>
<td>0.851</td>
<td>0.850</td>
<td>0.883</td>
<td>0.865</td>
<td>0.800</td>
<td>0.902</td>
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<tr>
<td>Calcium (%)</td>
<td>0.857</td>
<td>0.921</td>
<td>1.21</td>
<td>0.976</td>
<td>0.926</td>
<td>1.01</td>
<td>0.969</td>
</tr>
<tr>
<td>Phosphorus Content (%)</td>
<td>0.718</td>
<td>0.748</td>
<td>0.766</td>
<td>0.750</td>
<td>0.729</td>
<td>0.691</td>
<td>0.710</td>
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<tr>
<td>Nonphytate Phosphorus (%)</td>
<td>0.473</td>
<td>0.508</td>
<td>0.526</td>
<td>0.508</td>
<td>0.485</td>
<td>0.465</td>
<td>0.453</td>
</tr>
</tbody>
</table>

1 Metabolizable Energy and Available Phosphorus were based on Agristat values as suggested by M. Donohue. 2013. The Challenges in Feeding Broilers in Times of High and Volatile Feed Ingredient Costs: How to Cover the Costs?, 2013 Mid-Atlantic Nutrition Conference proceedings. Calcium values were adjusted to a 2.1:1 of Calcium to Available phosphorus.

2 Digestible amino acids were based on values suggested by P. B. Tillman and W.A. Dozier. 2013. Current Amino Acid Considerations for Broilers: Requirements, Ratios, Economics for 8 – 14 day broilers. www.thepoultryfederation.com

3 The percent Nonphytate Phosphorus (NPP) was calculated using the following equation: % NPP = % total Phosphorus – (0.282 * % Phytic Acid)
Table 2. Effects of Modification within Diet and Feed Manufacture Concern Areas that are Notorious for Decreasing Pellet Quality.

<table>
<thead>
<tr>
<th>Feed Milling Concern¹</th>
<th>Level of Concern</th>
<th>New Holman Test Pellet Survivability (%)</th>
<th>Pellets (%)</th>
<th>Crumbles (%)</th>
<th>Fines (%)</th>
<th>Particle Size (µm)</th>
<th>Conditioner Energy (KWH/MT)</th>
<th>Pellet Mill Energy (KWH/MT)</th>
<th>Production Rate (MT/H)</th>
<th>Hot Pellet Temperature (°F)</th>
</tr>
</thead>
<tbody>
<tr>
<td>DDGS</td>
<td>Low²</td>
<td>90.1b⁶</td>
<td>89.9b</td>
<td>6.5c</td>
<td>3.5</td>
<td>4372b⁵</td>
<td>0.94</td>
<td>12.5</td>
<td>1.07</td>
<td>178.3⁵</td>
</tr>
<tr>
<td></td>
<td>High¹</td>
<td>91.8b⁶</td>
<td>90.3b</td>
<td>5.9d</td>
<td>3.6</td>
<td>4586⁶</td>
<td>1.01</td>
<td>12.6</td>
<td>1.05</td>
<td>178.6⁵</td>
</tr>
<tr>
<td>Rock Phosphate</td>
<td>Low⁴</td>
<td>91.7b⁶</td>
<td>90.2b</td>
<td>5.9d</td>
<td>3.7</td>
<td>4583⁶</td>
<td>0.90</td>
<td>12.0</td>
<td>1.08</td>
<td>178.3⁵</td>
</tr>
<tr>
<td></td>
<td>High¹</td>
<td>81.9d⁴</td>
<td>85.1d</td>
<td>10.3a</td>
<td>4.5</td>
<td>3888c⁴</td>
<td>0.88</td>
<td>11.6</td>
<td>1.07</td>
<td>177.6⁵</td>
</tr>
<tr>
<td>Mixer Added Fat</td>
<td>Low⁶</td>
<td>95.3a⁶</td>
<td>93.2a</td>
<td>4.1d</td>
<td>2.5</td>
<td>4498b⁶</td>
<td>0.90</td>
<td>13.4</td>
<td>1.06</td>
<td>179.6⁵</td>
</tr>
<tr>
<td></td>
<td>High⁷</td>
<td>86.4ad⁶</td>
<td>86.7ad</td>
<td>8.6b</td>
<td>4.4</td>
<td>4180⁶</td>
<td>0.86</td>
<td>11.3</td>
<td>1.08</td>
<td>176.7⁵</td>
</tr>
<tr>
<td>Conditioning</td>
<td>Low⁸</td>
<td>92.8b⁸</td>
<td>89.8b</td>
<td>6.4e</td>
<td>3.6</td>
<td>4496b⁸</td>
<td>0.90</td>
<td>11.8</td>
<td>1.07</td>
<td>180.0⁵</td>
</tr>
<tr>
<td></td>
<td>High⁹</td>
<td>88.6ac⁸</td>
<td>88.5ac</td>
<td>7.5bc</td>
<td>3.9</td>
<td>4220⁸</td>
<td>0.86</td>
<td>12.6</td>
<td>1.07</td>
<td>165.3⁵</td>
</tr>
</tbody>
</table>

Treatment p-value
- 0.0010
- 0.0003
- 0.0002
- 0.1162
- 0.0151
- 0.0006
- 0.7447
- 0.7082
- 0.0001

Treatment SEM¹⁰
- 1.58
- 0.83
- 0.60
- 0.44
- 123.55
- 0.06
- 0.24
- 0.01
- 1.43

Feed Milling Concern Means

| DDGS                  | ---- | 91.3 | 90.1 | 6.2 | 3.5 | 4479 | 0.97 | 12.5 | 1.06 | 178.5 |
| Rock Phosphate        | ---- | 86.8 | 87.6 | 8.1 | 4.1 | 4235 | 0.89 | 11.8 | 1.07 | 177.2 |
| Mixer Added Fat       | ---- | 90.9 | 90.0 | 6.4 | 3.4 | 4339 | 0.88 | 12.4 | 1.07 | 178.2 |
| Conditioning          | ---- | 90.7 | 89.1 | 7.0 | 3.7 | 4358 | 0.88 | 12.2 | 1.07 | 172.5 |

Feed Milling Concern SEM¹⁰
- 1.14
- 0.73
- 0.55
- 0.36
- 89.35
- 0.04
- 0.50
- 0.01
- 1.37

Level of Concern Means

| ----                  | Low   | 92.7 | 90.8 | 5.7 | 3.3 | 4487 | 0.91 | 12.4 | 1.07 | 179.0 |
|                       | High  | 87.2 | 87.6 | 8.1 | 4.1 | 4218 | 0.90 | 12.0 | 1.07 | 174.6 |

Level of Concern SEM¹⁰
- 0.80
- 0.51
- 0.39
- 0.25
- 63.18
- 0.03
- 0.35
- 0.01
- 0.97

Probability Values

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<tr>
<th>Feed Milling Concern</th>
<th>0.0439</th>
<th>0.1024</th>
<th>0.1177</th>
<th>0.5956</th>
<th>0.3260</th>
<th>0.3908</th>
<th>0.7614</th>
<th>0.7954</th>
<th>0.0202</th>
</tr>
</thead>
<tbody>
<tr>
<td>Level of Concern</td>
<td>0.0002</td>
<td>0.0005</td>
<td>0.0006</td>
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<td>0.0084</td>
<td>0.8675</td>
<td>0.4228</td>
<td>0.5036</td>
<td>0.0055</td>
</tr>
<tr>
<td>Concern * Level</td>
<td>0.0144</td>
<td>0.0133</td>
<td>0.0104</td>
<td>0.3151</td>
<td>0.0202</td>
<td>0.7713</td>
<td>0.2484</td>
<td>0.5600</td>
<td>0.0057</td>
</tr>
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</table>

¹Feed milling concerns were exasperated by the use of 16.6% moisture corn
²Dietary Distillers Dry Grains and Soluble inclusion of low concern = 2%, ³Dietary Distillers Dry Grains and Soluble inclusion of high concern = 8%
⁴Dietary rock phosphorus inclusion of low concern = 1.63%, ⁵Dietary rock phosphorus inclusion of high concern = 0.31%
⁶Mixer added fat of low concern = 0.5%, ⁷Mixer added fat of high concern = 2.5%
⁸Conditioning temperature of low concern = 175°F, ⁹Conditioning temperature of high concern = 160
¹⁰Standard Error of the Mean
Table 3. Effects of Modification within Diet and Feed Manufacture Concern Areas that are Notorious for Decreasing Pellet Quality.

<table>
<thead>
<tr>
<th>Feed Milling Concern</th>
<th>Level of Concern</th>
<th>New Holman Test Pellet Survivability (%)</th>
<th>Pellets (%)</th>
<th>Crumbles (%)</th>
<th>Fines (%)</th>
<th>Particle Size (µm)</th>
<th>Conditioner Energy (KWH/MT)</th>
<th>Pellet Mill Energy (KWH/MT)</th>
<th>Production Rate (MT/H)</th>
<th>Hot Pellet Temperature (°F)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mixer Added Fat</td>
<td>Low¹</td>
<td>95.3</td>
<td>93.2</td>
<td>4.1</td>
<td>2.5</td>
<td>4498</td>
<td>0.90</td>
<td>13.4</td>
<td>1.06</td>
<td>179.6</td>
</tr>
<tr>
<td></td>
<td>High²</td>
<td>86.4</td>
<td>86.7</td>
<td>8.6</td>
<td>4.4</td>
<td>4180</td>
<td>0.86</td>
<td>11.3</td>
<td>1.08</td>
<td>176.7</td>
</tr>
<tr>
<td>Conditioning</td>
<td>Low³</td>
<td>92.8</td>
<td>89.8</td>
<td>6.4</td>
<td>3.6</td>
<td>4496</td>
<td>0.90</td>
<td>11.8</td>
<td>1.07</td>
<td>179.7</td>
</tr>
<tr>
<td></td>
<td>High⁴</td>
<td>88.6</td>
<td>88.5</td>
<td>7.5</td>
<td>3.9</td>
<td>4220</td>
<td>0.86</td>
<td>12.6</td>
<td>1.07</td>
<td>165.3</td>
</tr>
<tr>
<td>DDGS</td>
<td>Low⁵</td>
<td>90.8</td>
<td>89.9</td>
<td>6.5</td>
<td>3.5</td>
<td>4372</td>
<td>0.94</td>
<td>12.5</td>
<td>1.07</td>
<td>178.3</td>
</tr>
<tr>
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<td>High⁶</td>
<td>91.8</td>
<td>90.3</td>
<td>5.9</td>
<td>3.6</td>
<td>4586</td>
<td>1.00</td>
<td>12.6</td>
<td>1.05</td>
<td>178.6</td>
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<tr>
<td>Rock Phosphate</td>
<td>Low⁷</td>
<td>91.7</td>
<td>90.2</td>
<td>5.9</td>
<td>3.7</td>
<td>4583</td>
<td>0.90</td>
<td>13.4</td>
<td>1.08</td>
<td>178.3</td>
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<tr>
<td></td>
<td>High⁸</td>
<td>81.9</td>
<td>85.1</td>
<td>10.3</td>
<td>4.5</td>
<td>3888</td>
<td>0.88</td>
<td>11.6</td>
<td>1.07</td>
<td>177.6</td>
</tr>
</tbody>
</table>

Contrast Probability Values

<table>
<thead>
<tr>
<th></th>
<th>Mixer Added Fat (Low vs. High Concern)</th>
<th>Conditioning (Low vs. High Concern)</th>
<th>DDGS (Low vs. High Concern)</th>
<th>Rock Phosphate (Low vs. High Concern)</th>
</tr>
</thead>
<tbody>
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<td>0.1702</td>
<td>0.7368</td>
<td>0.0027</td>
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<td>0.4548</td>
<td>0.8089</td>
<td>0.0080</td>
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<td>0.4057</td>
<td>0.6366</td>
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<td></td>
<td>0.0029</td>
<td>0.6541</td>
<td>0.7469</td>
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<td></td>
<td>0.0779</td>
<td>0.1235</td>
<td>0.2289</td>
<td>0.0004</td>
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<td></td>
<td>0.6423</td>
<td>0.6619</td>
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<td></td>
<td>0.0099</td>
<td>0.3334</td>
<td>0.9222</td>
<td>0.6495</td>
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<tr>
<td></td>
<td>0.5111</td>
<td>0.9511</td>
<td>0.2342</td>
<td>0.4823</td>
</tr>
<tr>
<td></td>
<td>0.1525</td>
<td>0.0001</td>
<td>0.8540</td>
<td>0.7256</td>
</tr>
</tbody>
</table>

¹Mixer added fat of low concern = 0.5%, ²Mixer added fat of high concern = 2.5%
³Conditioning temperature of low concern = 175°F, ⁴Conditioning temperature of high concern = 160°F
⁵Dietary Distillers Dry Grains and Soluble inclusion of low concern = 2%, ⁶Dietary Distillers Dry Grain and Soluble inclusion of high concern = 8%
⁷Dietary rock phosphorus inclusion of low concern = 1.63%, ⁸Dietary rock phosphorus inclusion of high concern = 0.31%
Table 4. Analyzed nutrients of corn, porcine MBM, and corn DDGS.

<table>
<thead>
<tr>
<th>Nutrient Analysis¹</th>
<th>Corn²</th>
<th>Porcine Meat and Bone Meal</th>
<th>Corn DDGS</th>
</tr>
</thead>
<tbody>
<tr>
<td>Moisture Content (%)</td>
<td>16.6</td>
<td>8.94</td>
<td>12.1</td>
</tr>
<tr>
<td>Protein Content (%)</td>
<td>7.29</td>
<td>48.6</td>
<td>28.7</td>
</tr>
<tr>
<td>Fat Content (%)</td>
<td>3.86</td>
<td>11.3</td>
<td>9.29</td>
</tr>
<tr>
<td>Crude Fiber Content (%)</td>
<td>1.84</td>
<td>1.17</td>
<td>8.15</td>
</tr>
<tr>
<td>Ash Content (%)</td>
<td>1.78</td>
<td>22.2</td>
<td>4.26</td>
</tr>
<tr>
<td>Calculated Nitrogen Free Extract³</td>
<td>68.63</td>
<td>7.79</td>
<td>37.5</td>
</tr>
</tbody>
</table>

¹Determined on samples “As Is”

²The particle size of the corn used in this study was found to be 834.22 microns.

³Nitrogen Free Extract percentage was calculated by adding moisture, protein, fat, fiber, and ash content and then subtracting from 100.
Comparison of a transgenic phytase corn and a granulated bacterial phytase in pelleted feed on 21-day broiler performance

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¹Division of Animal and Nutritional Sciences, West Virginia University, Morgantown, West Virginia, 26506-6108 and †Center for Expertise and Research in Nutrition, Adisseo France, S.A.S.

¹ Corresponding author: jsmoritz@mail.wvu.edu
Primary Audience: Nutritionists, Feed Mill Managers, Researchers

SUMMARY

Phytase use in feed for broiler chickens reduces formulation cost, improves bird performance, and reduces environmental impact. Novel phytase products may demonstrate greater advantages compared to established phytase products. The objective of the current study was to compare dose effects of a novel transgenic phytase corn product and an established granulated bacterial phytase in pelleted feed on 21-d broiler performance and tibia mineralization. Feed formulations were based on the phytase activity provided by the manufacturer and provided a calculated activity of 500, 1,500, and 6,000 FTU/kg for each phytase product. In addition, a positive and negative control diet based on non-phytate phosphorus level were formulated. All eight experimental treatments were steam conditioned at 70°C for 10sec and extruded through a 4.7 x 38 mm pellet die. Pellets were crumbled to a similar size and fed to 12 replicate pens of 10 straight-run Hubbard x Cobb broiler chicks for 21-d. Diets were analyzed for post pellet phytase activity and broiler performance and tibia mineralization was analyzed using a 3 (dose) x 2 (phytase product) factorial arrangement within a randomized complete block design as well as a multiple comparison of all eight dietary treatments. Positive and negative control formulations produced relative differences in live weight gain, ending bird weight, and feed conversion ratio as expected (P<0.05). Main effects of dose and phytase product significantly affected live weight gain, ending bird weight, and feed conversion ratio. The granulated bacterial phytase was superior to the transgenic phytase corn product and the 6,000 FTU/kg dose was superior to the 500 and 1,500 FTU/kg doses (P<0.05). Phytase analytical activity was lower than the calculated activity for the transgenic phytase corn product. The manufacturer activity suggested for the transgenic phytase corn may have been...
overestimated and/or the thermal stability of the product may have been low. These factors likely contributed to reduced efficacy of the transgenic phytase corn.

Keywords: phytase, transgenic corn, broiler, FCR

DESCRIPTION OF PROBLEM

The major storage form of phosphorus (P) in plant materials is known as phytate (myo-inositol 1,2,3,4,5,6-hexakis dihydrogen phosphate or IP6) which is largely indigestible to monogastric animals [1-2]. With the majority of poultry being fed plant based diets, the amount of available P in the diets is a major concern [3]. Other concerns that arise with respects to phytate are the consequences its presence has on the availability of additional nutrients, such as minerals (i.e. Ca, Mg, K) and amino acids [1, 4]. In combination with these minerals, IP6 will form a complex salt which is known as phytin [1]. The use of phytase products has been found to help circumvent these issues by making a portion of the indigestible P available to both poultry and swine [4-5].

Phytase products are historically microbially expressed products that are presented in either a granulated, coated, or liquid form. Phytase products have also been found to help improve broiler performance metrics when negative control diets (i.e. low phosphorus diets) such as feed intake, live weight gain, and feed efficiency [6]. As a possible substitute for these traditional, more established phytase products, transgenic phytase corn (TPC) products are being tested [7-12]. Denbow et al (1998) found that transformed soybeans and a commercial microbial phytase had similar effects on body weight gain, feed intake, and feed efficiency when included at an equal activity level [12]. Similar results were reported for corn-based phytase products [8].

Another cause of concern with respect to phytase inclusions is the dosage of phytase added to the mash feed. Normal accepted levels of phytase inclusions consist of 250 FTU/kg,
which is the point at which 1µmol of inorganic phosphate is released per minute from a 5.1mM sodium phytate in a citrate buffer with a pH of 5.5 at 37°C [13]. Researchers have also been investigating the utilization of a super dose of phytase levels in the feed. This occurs when phytase is fed at a level where the basic requirements for P, calcium, and sodium are exceeded [14]. Additionally, when super doses are fed, extra-phosphoric effects have been observed in conjunction with several other beneficial results [15-17]. Shirley and Edwards [16] found that in addition to phosphorus deficiencies being overcome, bird weight gain, feed intake, and feed:gain ratios all improved to levels equal to those of the control diets. While feeding these super doses is beneficial, the great variation within the phytase activity makes it harder to definitely specify if the feed is in fact a super dose. Past research has shown that different methodologies for determining phytase activity can result in different phytase activities within the same laboratory [18].

Pelleting feed in the broiler industry is a long established process [19-23]. There are many advantages that are associated with the pelleting of feeds including improved performance and bulk density as well as decreased feed wastage and selective feeding [24]. Additionally, past research has shown that increasing the quality of the pellets provided to the bird will improve feed conversion ratios while decreasing feed intake [54-31]. Due to the prevalence of pelleting feed in the industry, it is imperative that any feed additive, i.e. phytases, are thermally stable enough to withstand the pelleting process [18].

Therefore the objective of the current study was to compare dose effects of a novel transgenic phytase corn product and an established granulated bacterial phytase in pelleted feed on 21-d broiler performance and tibia mineralization. The phytase treatments consisted of inclusions of 500, 1500, and 6000 FTU/g of either a transgenic phytase corn or a granulated
phytase. Additionally, a positive and negative control diet was formulated and fed for comparison.

MATERIALS AND METHODS

Experimental Design

Experimental treatments were structured as a 3 x 2 factorial arrangement within a randomized complete block design with a positive and negative control. The main effects consisted of the three inclusion doses of phytase and two phytase products. Table 1 may better describe the treatment differences. Each treatment was replicated 12 times within the study. All diets were formulated to similar calculated nutrient values based on commercial [32] and research recommendations [33] (Table 2).

Diet Preparations and Feed Manufacture

Feed manufacture was conducted at the West Virginia University pilot feed mill and descriptive data was collected for manufacture efficiency and pellet quality. The feed was batched and mixed using a one-ton vertical screw Easy Automation Inc. mixer [34]. Master batches of feed were mixed and split for the negative control and all phytase treatments so that all basal ingredients would be similar among treatments. The appropriate amounts of phytase product (either the transgenic phytase corn product or a granulated bacterial phytase (GBP) derived from a Buttiauxella species) was calculated based on the manufacturer’s provided phytase activity; sand was supplemented to attain the required inclusions for each treatment. A separate batch was completed for the positive control treatment. The treatments were steam conditioned using a 1.3 x 0.31 m short-term (10 s) California Pellet Mill conditioner at 70°C. The feed was then extruded through a 4.7 x 38 mm pellet die driven by a 40-horsepower California Pellet Mill [35]. Following pelleting, all treatments were ground via a roller mill before feeding.
Samples were collected post pelleting from the cooler deck to assess pellet quality through the New Holmen Pellet Tester (NHPT) (Table 3) [36]. Crumble particle size was determined for each treatment to obtain a clear description of the feed the birds were presented with (Table 3). Additionally, the hot pellet temperature, average pellet mill motor amperage, and production rate were recorded for each treatment (Table 3).

**Enzyme Activity**

Pelleted feed samples were collected after cooling and were sent to a commercial laboratory [37] for phytase activity analysis. The phytase analysis was based on the AOAC methodology AOAC 2000.12 [38].

**Growth Performance**

A total of 960 one-day-old, straight run Hubbard x Cobb broilers were obtained from a commercial hatchery [39]. Ten birds were then assigned at random to 1 of 96 raised wire cages (30.48 cm x 50.8 cm) located within two rooms of equal size which are cross-ventilated negative-pressure rooms. Water and feed were provided ad libitum to the birds via either a suspended nipple drinker located in the back of the cage or an open trough located at the front of the cage, respectively. Schedules for lighting, temperature, and ventilation mimicked those of a commercial integrator. Pen weights were obtained prior to placement and were kept within the range of +/- 100 grams of the average. Pen weights were again recorded at d 21 in order to measure the growth variables feed intake, live weight gain, and feed conversion ratio (adjusted for mortality) (Table 4). All birds were euthanized via cervical dislocation. After euthanization, the left tibia was excised from all birds in each pen for 8 replicates for the future calculation of tibia ash. Bird rearing methods were in compliance with the West Virginia University Animal Care and Use Committee Guidelines.
Statistical Analysis

Performance variables were analyzed using the 3 (inclusion level) x 2 (phytase product) factorial arrangement in a randomized complete block. Each block consisted of one replicate of each treatment with each treatment being replicated 12 times. The experimental unit consisted of one cage of 10 broilers. Analysis was completed using ANOVA with means being further explored using a Fisher’s LSD multiple comparison test when means were significant. Additionally, a multiple comparison of all eight dietary treatments was performed. Significance was determined at P < 0.05.

RESULTS AND DISCUSSION

Descriptive feed data is presented in Table 3, and the main effects and multiple comparisons are presented in Table 4. Additionally, phytase activity in the feed samples is listed in Table 2. There were no interactions between enzyme and dose observed for the treatments (Table 4).

Feed Manufacture

Results obtained for hot pellet temperature, average pellet mill motor amperage, production rate, pellet survivability, and crumble particle size all remained within close proximity of the other treatments (Table 3). The hot pellet temperature recorded for all treatments stayed within the range of 75.2-76.2°C. This is notable since the conditioning temperature utilized for the study remained at 70°C, likely reducing water for lubrication and increasing friction in the pellet die. The authors speculate that this result may have affected the activity level of the phytase products in the six phytase-containing experimental diets (Table 2).

Enzyme Activity
Phytase activity data showed that the TPC treatments demonstrated phytase activity levels that were less than the calculated values (Table 2). However, the GBP treatments demonstrated levels that were close to the calculated values (Table 2).

**Growth Performance**

Overall multiple comparisons showed significant results for live weight gain (LWG) ($P = 0.0001$), ending bird weight ($P = 0.0001$), and feed conversion ratio (FCR) ($P = 0.0001$, Table 4). Onyango *et al.* [40] demonstrated that weight gain and gain to feed ratios increase significantly when a phytase product is included in diets that are deficient in P. The current study also showed that there was no significant difference in feed intake between treatments ($P > 0.05$, Table 4).

Significant results demonstrated that the GBP increased the LWG ($P = 0.0069$) and ending bird weight ($P = 0.0025$) more efficiently when compared to the TPC (Table 4). Nyannor and Adeola [8] reported significant results when a TPC was compared to a commercial product. Similar results were reported by Denbow *et al.* [12]. Additionally, FCR ($P = 0.0010$) decreased significantly in treatments utilizing the GBP when compared to the treatments utilizing the TPC (Table 4). These results do not agree with past research [8, 12], however, the results reported in both studies only reported data for weeks two and three (d 7-21).

As dose level increased, a significant increase in LWG ($P = 0.0002$) and ending bird weight ($P = 0.0002$) was observed, while a significant decrease in FCR ($P = 0.0001$) was recorded across phytase products (Table 4). Shirley and Edwards [16] found that as phytase dosage increased, so did bird weight gain and the gain to feed ratio. Tang *et al.* found that increasing the inclusion of phytase causes an increase in body weight gain and feed intake and a decrease in FCR when compared to diets calculated to exhibit a full phosphorus content [6].
CONCLUSIONS AND APPLICATIONS

1. Feeding the granulated bacterial phytase produced superior broiler performance compared to the transgenic phytase corn product.

2. Diet incorporation of the calculated 6,000 FTU/kg dose improved performance above the 500 and 1,500 FTU/kg doses across phytase products.

REFERENCES AND NOTES


[32] Metabolizable Energy and Available Phosphorus were based on Agristat values as suggested by M. Donohue. 2013. The Challenges in Feeding Broilers in Times of High and Volatile Feed Ingredient Costs: How to Cover the Costs?. 2013 Mid-Atlantic Nutrition Conference proceedings. Calcium values were adjusted to a 2.1:1 of Calcium to Available phosphorus.


[34] MFP Vertical Mixer, Easy Automation Inc., Welcome, MN.


[36] Pellet quality was assessed one day following production using the New Holmen Portable Pellet Durability Tester, Lignotech USA, INC., Rothschild, WI. 100 g of pellets were sifted through No. 5 American Society for Testing and Materials (ASTM) screen and placed in holding chamber, blown for 30 s by a jet of air, then weighed, giving a direct read of pellet durability. Fine is removed during the blowing process.
[37] Eurofins Scientific, Des Moines, IA.

[38] AOAC. 2000. Method 2000.12: Phytase activity in feed: Colorimetric enzymatic method. In Official Methods of Analysis of AOAC International. 17th ed. Assoc. Off. Anal. Chem., Arlington, VA. A 50-g sample was extracted with 500 mL of distilled water containing 0.01% Tween 20. A 100-µL extract was diluted with 300 mL of acetate buffer until reaching pH 5.5. After preincubation of the diluted extract at 37°C, 0.8 mL of sodium phytate was added, and the sample was incubated for 30 min at 37°C. The reaction was stopped via addition of 0.8 mL of molybdate-vanadate stop reagent. A blank was included, and after its preincubation, the stop reagent was added before the addition of the substrate. The yellow complex was measured colorimetrically at 415 nm. The value obtained from the blank was then subtracted from the sample value, and the phosphate released was quantified with a phosphate standard curve.

[39] Pilgrims Pride Hatchery. Moorefield, WV.

Table 1. Treatment Structure

<table>
<thead>
<tr>
<th>Treatment #</th>
<th>Treatment Description</th>
<th>Treatment Specifications</th>
</tr>
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<tbody>
<tr>
<td>1</td>
<td>Positive Control</td>
<td>0.4% AP and 0.96% Ca</td>
</tr>
<tr>
<td>2</td>
<td>Negative Control</td>
<td>0.25% AP and 0.84% Ca</td>
</tr>
<tr>
<td>3</td>
<td>Negative Control + 500 FTU/kg TPC</td>
<td>0.25% AP and 0.84% Ca</td>
</tr>
<tr>
<td>4</td>
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<td>0.25% AP and 0.84% Ca</td>
</tr>
<tr>
<td>5</td>
<td>Negative Control + 6,000 FTU/kg TPC</td>
<td>0.25% AP and 0.84% Ca</td>
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<tr>
<td>6</td>
<td>Negative Control + 500 FTU/kg GBP</td>
<td>0.25% AP and 0.84% Ca</td>
</tr>
<tr>
<td>7</td>
<td>Negative Control + 1,500 FTU/kg GBP</td>
<td>0.25% AP and 0.84% Ca</td>
</tr>
<tr>
<td>8</td>
<td>Negative Control + 6,000 FTU/kg GBP</td>
<td>0.25% AP and 0.84% Ca</td>
</tr>
</tbody>
</table>
Table 2. Diet Formulations and Calculated Nutrients

<table>
<thead>
<tr>
<th>Ingredients</th>
<th>Positive Control (%)</th>
<th>Negative Control (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Corn</td>
<td>51.35</td>
<td>52.56</td>
</tr>
<tr>
<td>Soybean Meal (48%)</td>
<td>35.03</td>
<td>34.88</td>
</tr>
<tr>
<td>Corn DDGS</td>
<td>5.00</td>
<td>5.00</td>
</tr>
<tr>
<td>Soybean Oil</td>
<td>4.01</td>
<td>3.64</td>
</tr>
<tr>
<td>Limestone</td>
<td>1.49</td>
<td>1.63</td>
</tr>
<tr>
<td>DL-Methionine</td>
<td>0.45</td>
<td>0.45</td>
</tr>
<tr>
<td>Dicalcium Phosphate</td>
<td>1.45</td>
<td>0.64</td>
</tr>
<tr>
<td>Salt</td>
<td>0.24</td>
<td>0.24</td>
</tr>
<tr>
<td>Vitamin Mineral Premix</td>
<td>0.25</td>
<td>0.25</td>
</tr>
<tr>
<td>Sodium Bicarbonate</td>
<td>0.15</td>
<td>0.15</td>
</tr>
<tr>
<td>Lysine</td>
<td>0.19</td>
<td>0.19</td>
</tr>
<tr>
<td>Threonine</td>
<td>0.14</td>
<td>0.14</td>
</tr>
<tr>
<td>Sand/Phytase1</td>
<td>0.24</td>
<td>0.24</td>
</tr>
</tbody>
</table>

Calculated Nutrients

<table>
<thead>
<tr>
<th>Nutrient</th>
<th>Treatment 1</th>
<th>Treatment 2</th>
<th>Treatment 3</th>
<th>Treatment 4</th>
<th>Treatment 5</th>
<th>Treatment 6</th>
<th>Treatment 7</th>
<th>Treatment 8</th>
</tr>
</thead>
<tbody>
<tr>
<td>ME (kcal/lb)</td>
<td>1361</td>
<td>1361</td>
<td>22.00</td>
<td>22.00</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>C.P. (%)</td>
<td>1.20</td>
<td>1.20</td>
<td>0.54</td>
<td>0.54</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Dig. Lysine 3 (%)</td>
<td>1.10</td>
<td></td>
<td>1.10 (0.90 minimum)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Dig. Methionine (%)</td>
<td>0.84</td>
<td>0.84</td>
<td>0.22</td>
<td>0.22</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Calcium (%)</td>
<td>0.96</td>
<td></td>
<td></td>
<td></td>
<td>0.96</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Available P (%)</td>
<td>0.40 (0.46 Agristat average)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Sodium (%)</td>
<td>0.17</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Analyzed Nutrients

<table>
<thead>
<tr>
<th>Nutrient</th>
<th>Treatment 1</th>
<th>Treatment 2</th>
<th>Treatment 3</th>
<th>Treatment 4</th>
<th>Treatment 5</th>
<th>Treatment 6</th>
<th>Treatment 7</th>
<th>Treatment 8</th>
</tr>
</thead>
<tbody>
<tr>
<td>Calcium (%)</td>
<td>1.01</td>
<td>0.913</td>
<td>0.863</td>
<td>0.989</td>
<td>0.942</td>
<td>0.955</td>
<td>1.00</td>
<td>0.844</td>
</tr>
<tr>
<td>Non-Phytate Phosphorous4 (%)</td>
<td>0.347</td>
<td>0.268</td>
<td>0.241</td>
<td>0.229</td>
<td>0.23</td>
<td>0.235</td>
<td>0.247</td>
<td>0.276</td>
</tr>
<tr>
<td>Phytase (FTU/g)</td>
<td>350</td>
<td>670</td>
<td>2,500</td>
<td>490</td>
<td>1,500</td>
<td>7,300</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

1 Phytase Activity (FTU/g): TPC = 2,100 and GBP = 2,600; Particle Size (µm): TPC = 740 and GBP = 497
2 Metabolizable Energy and Available Phosphorus were based on Agristat values as suggested by M. Donohue. 2013. The Challenges in Feeding Broilers in Times of High and Volatile Feed Ingredient Costs: How to Cover the Costs?. 2013 Mid-Atlantic Nutrition Conference proceedings. Available Phosphorus in the NC was reduced by 0.21 of the US average. Calcium values were adjusted to a 2.4:1 of Calcium to Available phosphorus for NC + 0.15% aP via DCP.
3 Digestible amino acids were based on the digestible lysine value (1.2%) suggested by P. B. Tillman and W.A. Dozier. 2013. Current Amino Acid Considerations for Broilers: Requirements, Ratios, Economics. www.thepoultryfederation.com for 8 – 14 day broilers. Digestible amino acid to digestible lysine ratios followed further recommendations of this communication (45 methionine, 70 threonine, 16 tryptophan).
4 The percent Non-Phytate Phosphorus (NPP) was calculated using the following equation: % NPP = % Total Phosphorus – (0.282 * % Phytic Acid)
Table 3: Descriptive feed manufacture data of dietary treatments that were steam conditioned at 70°C in a 0.31 x 1.3 m conditioner for 10 sec and extruded through a 4.7 x 38 mm pellet die.

<table>
<thead>
<tr>
<th>Enzyme</th>
<th>Dose</th>
<th>Formulated Available P and Total Ca</th>
<th>Hot Pellet Temperature(^1) (°C)</th>
<th>Average Pellet Mill Motor Amperage</th>
<th>Production Rate (Tonne/hr)</th>
<th>Pellet Survivability due to NHPT(^2) (%)</th>
<th>Crumble Particle Size(^3) (µm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>No</td>
<td>No</td>
<td>0.40 and 0.96</td>
<td>76.2</td>
<td>19.85</td>
<td>0.741</td>
<td>36.86</td>
<td>895.9</td>
</tr>
<tr>
<td>No</td>
<td>No</td>
<td>0.25 and 0.84</td>
<td>76.2</td>
<td>19.2</td>
<td>0.803</td>
<td>34.21</td>
<td>1175.0</td>
</tr>
<tr>
<td>TPC</td>
<td>500 FTU/kg</td>
<td>0.25 and 0.84</td>
<td>76.2</td>
<td>18.8</td>
<td>0.853</td>
<td>30.025</td>
<td>1207.3</td>
</tr>
<tr>
<td>TPC</td>
<td>1,500 FTU/kg</td>
<td>0.25 and 0.84</td>
<td>76.1</td>
<td>19.25</td>
<td>0.803</td>
<td>41.33</td>
<td>981.9</td>
</tr>
<tr>
<td>TPC</td>
<td>6,000 FTU/kg</td>
<td>0.25 and 0.84</td>
<td>75.9</td>
<td>18.1</td>
<td>0.853</td>
<td>29.175</td>
<td>1029.9</td>
</tr>
<tr>
<td>GBP</td>
<td>500 FTU/kg</td>
<td>0.25 and 0.84</td>
<td>75.2</td>
<td>19.55</td>
<td>0.804</td>
<td>33.825</td>
<td>1080.8</td>
</tr>
<tr>
<td>GBP</td>
<td>1,500 FTU/kg</td>
<td>0.25 and 0.84</td>
<td>75.2</td>
<td>19.05</td>
<td>0.847</td>
<td>30.09</td>
<td>1033.9</td>
</tr>
<tr>
<td>GBP</td>
<td>6,000 FTU/kg</td>
<td>0.25 and 0.84</td>
<td>75.6</td>
<td>19.3</td>
<td>0.853</td>
<td>26.05</td>
<td>1053.8</td>
</tr>
</tbody>
</table>

\(^1\)Hot pellet temperature was determined on pellets directly following extrusion from the die. Pellets were collected into an insulated container and temperature was measured using a thermocouple thermometer and an 80PK-24 temperature probe.

\(^2\)Measurements of PDI were obtained using the New Holmen Pellet Tester, where 100 g pelleted samples are subjected to air flow within a perforated chamber for 30 s.

\(^3\)Particle size was determined with a Ro-Tap particle size analyzer model RX-29 Type 110V 60H2.
Table 4: Statistical Comparison of all dietary treatments as well as the factorial arrangement of treatments on 21 D Hubbard x Cobb straight-run broiler performance.

<table>
<thead>
<tr>
<th>Enzyme</th>
<th>Dose</th>
<th>Formulated Available P and Total Ca</th>
<th>Starting Pen Weight (kg)</th>
<th>Feed Intake / bird (kg)</th>
<th>Live Weight Gain / bird (kg)</th>
<th>Ending Bird Weight (kg)</th>
<th>FCR(^1) (kg/kg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>No</td>
<td>No</td>
<td>0.40 and 0.96</td>
<td>0.432</td>
<td>1.004</td>
<td>0.744(^{bc})</td>
<td>0.784(^{bc})</td>
<td>1.35(^{de})</td>
</tr>
<tr>
<td>No</td>
<td>No</td>
<td>0.25 and 0.84</td>
<td>0.435</td>
<td>0.996</td>
<td>0.699(^{d})</td>
<td>0.741(^{e})</td>
<td>1.42(^{ab})</td>
</tr>
<tr>
<td>TPC</td>
<td>500 FTU/kg</td>
<td>0.25 and 0.84</td>
<td>0.433</td>
<td>1.008</td>
<td>0.705(^{d})</td>
<td>0.748(^{de})</td>
<td>1.43(^{a})</td>
</tr>
<tr>
<td>TPC</td>
<td>1,500 FTU/kg</td>
<td>0.25 and 0.84</td>
<td>0.435</td>
<td>1.023</td>
<td>0.721(^{cd})</td>
<td>0.755(^{cde})</td>
<td>1.42(^{ab})</td>
</tr>
<tr>
<td>TPC</td>
<td>6,000 FTU/kg</td>
<td>0.25 and 0.84</td>
<td>0.434</td>
<td>1.062</td>
<td>0.762(^{ab})</td>
<td>0.804(^{ab})</td>
<td>1.39(^{bc})</td>
</tr>
<tr>
<td>GBP</td>
<td>500 FTU/kg</td>
<td>0.25 and 0.84</td>
<td>0.435</td>
<td>1.039</td>
<td>0.733(^{bcd})</td>
<td>0.777(^{bcd})</td>
<td>1.42(^{ab})</td>
</tr>
<tr>
<td>GBP</td>
<td>1,500 FTU/kg</td>
<td>0.25 and 0.84</td>
<td>0.435</td>
<td>1.041</td>
<td>0.754(^{bc})</td>
<td>0.798(^{ab})</td>
<td>1.38(^{cd})</td>
</tr>
<tr>
<td>GBP</td>
<td>6,000 FTU/kg</td>
<td>0.25 and 0.84</td>
<td>0.435</td>
<td>1.053</td>
<td>0.791(^{a})</td>
<td>0.831(^{a})</td>
<td>1.33(^{a})</td>
</tr>
</tbody>
</table>

**ANOVA P-value**

|                | 0.4975 | 0.2218 | 0.0001 | 0.0001 | 0.0001 |

**Fisher’s LSD**

|                | --    | --     | 0.037  | 0.035  | 0.034  |

**SEM\(^2\)**

|                | 0.0013 | 0.0204 | 0.0130 | 0.0124 | 0.0120 |

**Marginal Means-Enzyme (TCP vs. GBP)**

<table>
<thead>
<tr>
<th>Enzyme</th>
<th>Feed Intake / bird (kg)</th>
<th>Live Weight Gain / bird (kg)</th>
<th>Ending Bird Weight (kg)</th>
<th>FCR(^1) (kg/kg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>TCP</td>
<td>0.434</td>
<td>0.729(^{b})</td>
<td>0.769(^{b})</td>
<td>1.41(^{a})</td>
</tr>
<tr>
<td>GBP</td>
<td>0.435</td>
<td>0.760(^{a})</td>
<td>0.802(^{a})</td>
<td>1.38(^{b})</td>
</tr>
<tr>
<td>SEM</td>
<td>0.0007</td>
<td>0.0077</td>
<td>0.0074</td>
<td>0.0078</td>
</tr>
</tbody>
</table>

**Marginal Means-Dose (500 FTU/kg vs. 1,500 FTU/kg vs. 6,000 FTU/kg)**

<table>
<thead>
<tr>
<th>Dose</th>
<th>Feed Intake / bird (kg)</th>
<th>Live Weight Gain / bird (kg)</th>
<th>Ending Bird Weight (kg)</th>
<th>FCR(^1) (kg/kg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>500 FTU/kg</td>
<td>0.433</td>
<td>0.719(^{b})</td>
<td>0.762(^{b})</td>
<td>1.42(^{a})</td>
</tr>
<tr>
<td>1,500 FTU/kg</td>
<td>0.435</td>
<td>0.738(^{b})</td>
<td>0.777(^{b})</td>
<td>1.40(^{a})</td>
</tr>
<tr>
<td>6,000 FTU/kg</td>
<td>0.435</td>
<td>0.777(^{a})</td>
<td>0.817(^{a})</td>
<td>1.36(^{b})</td>
</tr>
<tr>
<td>SEM</td>
<td>0.0009</td>
<td>0.0094</td>
<td>0.0091</td>
<td>0.0096</td>
</tr>
</tbody>
</table>

**Probability Values for Main Effects and the Interaction**

<table>
<thead>
<tr>
<th>Effect</th>
<th>Probability Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Enzyme</td>
<td>0.1928</td>
</tr>
<tr>
<td>Dose</td>
<td>0.6456</td>
</tr>
<tr>
<td>Enzyme x Dose</td>
<td>0.7855</td>
</tr>
</tbody>
</table>

\(^{1}\)FCR= Feed Conversion Ratio, corrected for mortality

\(^{2}\)SEM= Standard Error of the Mean
CONCLUSION

While the results of the studies conducted were interesting, they do leave some questions unanswered. Therefore, additional research should be conducted to further explore both the effects of diet ingredients and feed manufacture technique on pellet quality as well as the effects of varying doses of two phytase products on broiler performance. Additionally, future research could focus on the cost benefits, both for the producer and the consumer.

The feed manufacture study provided several different improvements that could be made during future studies as well as several follow-up studies that could be conducted. One major improvement that could be made to the study is to conduct the study during a different time of the year when the ambient temperatures are warmer. Another possible improvement that could be made to the study would be to replace the corn utilized in this study with a corn with a lower moisture content. A follow-up study could be conducted in which the feeds produced were fed to broiler chicks so that the effectiveness of the feed can be determined based on broiler performance.

The results of the phytase study left questions about how the results would have been different had the phytase activity of the transgenic phytase corn been closer to the calculated value provided by the manufacturer. If this study were to be repeated, the phytase inclusions should be determined based on analyzed phytase activity as opposed to the phytase activity provided by the manufacturer. Additionally, there are a couple of possible follow-up studies that could be conducted based on the current study. One possible follow-up study could further examine the effects of phytase inclusions by utilizing additional phytase products. Another possible follow-up study could utilize more phytase doses to examine the benefits found at each different dose.
Both studies conducted offered areas by which the costs associated with the production of poultry products can be reduced. However, neither study focused upon these areas. In the future, the effectiveness of the techniques explored at reducing costs could be examined and further explored to create the most cost effective processes. Additionally, the benefits of these techniques as seen by the consumer can be explored.

In conclusion, there are several take home messages that can be inferred. The main take home message from the feed manufacture study is that a greater appreciation of comprehensive formulation and manufacture technique effects are required since the data observed for the concern areas did not follow what literature or anecdotal information suggested. Additionally, this information could help pellet mill operators, nutritionists, and pelleting aid vendors better circumvent hurdles that are encountered during the pelleting process. The phytase study offered several messages which included that: 1) phytase products should have a guaranteed accurate analysis provided by the manufacturer and be thermally stable; 2) birds fed granulated bacterial phytase diets will perform better than birds fed a transgenic phytase corn diet; 3) higher doses of phytase included in the diet will improve broiler performance results; and 4) there are additional benefits beyond phosphorus liberation that can be obtained from feeding super-doses of phytases.
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Employment

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MASTER OF SCIENCE | SPRING 2015-CURRENT | WEST VIRGINIA UNIVERSITY
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EMPHASIS: POULTRY SCIENCE
GPA: 2.97

THESIS: THE EFFECTS OF FEED INGREDIENTS AND MANUFACTURE TECHNIQUE ON PELLET QUALITY AND PHYTASE INCLUSION LEVELS ON BROILER PERFORMANCE

BACHELOR OF SCIENCE | DECEMBER 2014 | WEST VIRGINIA UNIVERSITY
MAJOR: ANIMAL NUTRITIONAL SCIENCES
MINOR: BUSINESS ADMINISTRATION
OVERALL GPA: 3.00; ANIMAL SCIENCE GPA: 3.04; MINOR GPA: 3.15

Publications

PEER-REVIEWED MANUSCRIPTS


ABSTRACTS

Rigby, T. R., B. G. Glover, K. L. Foltz, and J. S. Moritz. 2016. Effects of modifying diet and feed manufacture concern areas that are notorious for decreasing pellet quality. (Accepted)
Research Experience

- Contract studies with Anitox and Adisseo
- Assisted with the following projects:
  - “The effects of varying short term steam conditioning temperatures on the mitigation of Enterococcus faecium 8459, a non-pathogenic surrogate of Salmonella”
  - “Influence of feed form (FF) and diet phase effects on 0-46 d broiler performance”
  - “Efficacy of a novel protease provided to broiler chicks in diets that vary in composition and degree of processing”
  - “Influence of feed form and diet phase effects on 46-53d broiler performance and processing”
  - “Evaluation of a rumen protected lysine product post pelleting using broiler chick apparent ileal digestibility and performance”
  - “Transgenic phytase corn and granulated phytase effects on mix uniformity, pellet quality, and thermal stability”
  - “Effects of feed form, environment, and caloric density on energy partitioning and subsequent broiler performance”
- Conducted the following studies:
  - “Effects of modification within diet and feed manufacture concern areas that are notorious for decreasing pellet quality”
  - “Comparison of a transgenic phytase corn and a granulated bacterial phytase in pelleted feed on 21-day broiler performance”

National Meeting Presentations:

- 2016 Poultry Science Association Annual Meeting (New Orleans, Louisiana) – Oral presentation titled “Effects of modification within diet and feed manufacture concern areas that are notorious for decreasing pellet quality”

Professional Development:


Teaching Experience

- Teaching assistant for Companion Animal Science – A&VS 275 (Fall 2015)
- Teaching assistant for Poultry Evaluation course – ANPR 339 (Spring 2016)

Extension Experience

- WV FFA CDE Poultry Judging Competition at WVU (June 2016)
- Presenter at Gilmer County Field Day (May 2016)
- Kiddie Days at the Farm (April 2016)

Academic Honors/Awards

- WV PROMISE Scholarship (2011-2014)
- WVU Storer Scholarship (2011-2014)