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Improving Broiler Performance Utilizing Modern Feed Additives

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ABSTRACT

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Niles R. Ridgeway

Modern broiler production strives to make modest improvements regarding broiler performance. This remains a goal as global population increases thusly increasing the quantity of an affordable, high quality source of protein. In recent years, an added stressor to achieving prior goals is mostly related to consciousness of environmental and consumer health. For decades, integrators have utilized minimal amounts of antibiotics as a barrier to most pathogens affecting the microbiome of a broiler's gastrointestinal tract. Regulatory efforts have now prohibited most of previous sub-therapeutic medicine and started an in-depth scope of broiler gut health and interaction with antibiotic alternatives. In an effort to provide beneficial bacteria in a challenged environment, broilers were fed Direct-Fed Microbials (DFM), notably *Bacillus subtilis*, to investigate performance improvements. Diets were formulated to meet bird requirements during specific age periods. 2,280 male Ross x Ross 708 broilers were placed on study for 42 days to evaluate live performance. Pens of 23 broilers were randomly assigned one of four dietary treatments; a control diet, and 3 diets compromised of the control and an additional top dressed DFM. A natural challenge was manifested by a combination of built-up litter and a weekly water spray to facilitate bacterial growth. Additionally, the diet was nutritionally limited. The results revealed that dietary treatments performed the same for most measurements. Live weight gain decreased in diets containing DFM2 or DFM3. Overall, broilers performed below industry expectations in each performance variable, suggesting the additive effect of nutritional deficit, floor conditions, and heat stress may have hindered opportunity for DFMs to perform or provide enough stimulus to generate expected results.

Additionally, transgenic grains were implemented into broiler diets to identify ability to liberate Ca and P by expressing phytase at two different concentrations. Different expressions resulted in volume discrepancies. Distribution throughout a mixer was of interest to identify potential for minimizing dietary inclusion thusly total cost. Phytase has long been utilized to combat P excretion in the poultry industry related to environmental concerns. Grain-expressed enzymes allow for a direct 1:1 replacement for the host grain. Adding exogenous enzymes without diluting dietary nutrients will be another means of improving performance by maximizing nutrient utilization. 2,304 male Ross x Ross 708 broilers were obtained and placed in pens of 24. A dietary factorial treatment structure was utilized for two corn-expressed phytase products at three doses. Additionally, a positive and negative control were used. Birds were selected randomly at day 21(n=5) and day 42(n=3), to be euthanized for tibia excision. Tibiae were collected, ashed and bone mineralization was determined to quantify liberation of additional P/Ca. Live performance was also measured. Results showed that a lower concentrated grain enzyme requires more volume and has more opportunity to distribute evenly during batching of a diet. This is reinforced by performance results that yield improved LWG for a product that requires more volume for a target dose.

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KEY

Chapter 1

1. United States of Agriculture – USDA
2. Coefficient of Variation – CV
3. No Antibiotics Ever – NAE
4. Veterinary Feed Directive – VFD
5. Direct-Fed Microbials – DFM
6. Phosphorus – P
7. Non-phytate Phosphrous – nPP
8. Available Phosphorus – aP

Chapter 2

1. Direct-fed microbial – DFM
2. Positive control – PC
3. Live weight gain – LWG
4. Feed conversion ratio – FCR
5. Antibiotics – AB
6. Gastrointestinal – GI
7. Veterinary feed directive- VFD
8. Dried distiller's grains w/solubles – DDGS
9. Phosphorus – P
10. Calcium – Ca
11. Horsepower – HP
12. Colony Forming Unit – CFU
13. Non phytate phosphorus – nPP
14. Pellet durability index – PDI
15. Modified pellet durability index – MPDI
16. New Holmen pellet tester – NHPT
17. Analysis of Variance – ANOVA
18. Mixer-added fat – MAF

Chapter 3

1. Corn-expressed phytase – CEP
2. Phosphorus – P
3. Feed intake – FI
4. Live weight gain – LWG
5. Feed conversion ratio – FCR
6. Coefficient of variation – CV
7. Fytase units – FTU
8. Metabolisable Energy - ME

CHAPTER 1: LITERATURE REVIEW

COMMERCIAL POULTRY PRODUCTION

An expanding population has projected a need for total food source to increase. Several agriculture models contribute to overall food capacity. Of the models, it is often believed that commercial poultry production is expected to meet the demands through the design of vertical integration. The vertical integration model has allowed poultry to annually remain the largest sector of the meat industry in terms of count and pounds produced.[1] Poultry consumption remains number one in pounds of consumption per capita each year in the United States. The United States Department of Agriculture (**USDA**) reports that 92.2 pounds of total chicken had been consumed on a per capita basis in 2017. Turkey adds an additional 16.4 pounds.[2] It trails only pork in total consumption on a global scale. It also remains the number one agricultural commodity in West Virginia, estimated to be a 400 million dollar enterprise in 2017. Total number of broilers (86 million per year) has dropped in recent years, but still ranks West Virginia 18th in the United States. Turkey production (3.7 million per year) places West Virginia at 14th.[3] As the global population increases, efforts to maximize broiler rearing efficiency will hold steadfast for years to come. Commercial production has made continuous improvements over the years in coordination with land-grant universities and federal agencies, USDA. The latter entities perform research in genetics, nutrition, biosecurity, and also management practices maximize current resources, while providing an affordable, highly nutritious protein.

FEED MANUFACTURE

Feeding an animal represents the major cost of animal production agriculture. Changes in feed form, specifically pelleting, have been known to increase feed efficiency whilst remaining cost-effective [4,5]. For this reason, more than 80% of non-ruminant feed is pelleted in the U.S.

[6]. Pelleting feed has many steps that vary accordingly, ultimately changing the quality of pellet produced. First, ground ingredients are mixed together with vitamins and minerals to provide a homogenous mash feed. This feed then is passed into a conditioning barrel that is direct fed with saturated steam to increase temperature and also pliability, through moisture addition, of the feed. As feed exits the conditioner, it falls into a press feeder that directs feed into a pellet press to be extruded. Extrusion occurs from the mash feed being pressed between rolls and an outer die, forcing the feed through holes in the die. As conditioned feed is extruded through the pellet die it is subjected to high friction and pressure. It is important to note that temperature increase across the die may be dependent on additional variables, such as rate of feed conveyance, die thickness, and diet composition [5].Once the compressed feed is extruded beyond the outer surface of the die, it takes on the form of a pellet. A stationary knife then cuts off the pellet at a desired length. Finally, pellets are conveyed to a cooling deck where moisture and temperature are both pulled off pellets to prevent growth of molds as well as potential nutrient dilutions from excess moisture.

Pelleting feed can provide performance improvements due to decreased feed wastage, decreased ingredient segregation, decreased energy expenditure, improved palatability, increased digestibility, and thermal modification of starch and protein [7].Pelleted diets improve broiler and swine performance when compared to mash diets [8-10]; however, the amount of benefit has been shown to be dependent upon pellet quality [5].

Nutrient availability has also been considered when looking at subjecting mash feed to the thermal processing technique of pelleting. Cutlip et al. conducted an experiment on temperature and pressure as they relate to nutrient availability finding that no differences could be found between unprocessed mash and conditioned pellets [11]. This contradicts Smith and

Circle that claim steam conditioning at high temperatures negatively affect nutrient availability, specifically amino acids found in soybean products [12]. The author [Cutlip et al.] attributes that his findings may be a result of a 3% inclusion of soybean oil at the mixer. Soybean oil has lubricating properties during the pelleting process. The lubrication increases throughput decreasing the amount of time feed may be subjected to friction during extrusion. Diets containing low mixer-added fat, subject feed to more time within the die, this can alter nutrient digestibility of ingredients that are not thermally stable such as amino acids noted by Smith and Circle.

MIX UNIFORMITY

An equal distribution of a diet's ingredients within a mixer can be coined "mix uniformity". A consistent mix of ingredients is important for the pelleting technique to provide an equal proportion of ingredients per pellet. Increasing importance arises during exogenous enzyme supplementation as these are typically miniscule amounts. A common measure of mix uniformity is mixer coefficient of variation (**CV**). A CV less than 10% is accepted to be a consistent mix. To determine CV, a marker is placed within the diet and added to the mixer. After a set mix time, a sample is taken to be analyzed for the marker placed into the diet. Pfost et al. stated criteria for marker selection[13]. Factors affecting mix uniformity usually involve, fill capacity, type of mixer, particle size of ingredients and mix time. McCoy et al. indicated improvements to mix uniformity improved feed efficiency of broilers [14].

ANTIBIOTIC ALTERNATIVES IN BROILER PRODUCTION

Modern poultry consumption continues to rise and meet the demands of consumers, but consumer perception often influences commercial rearing standards. Larger producers are

beginning to shift focus to antibiotic alternative production to meet the demand for 'No Antibiotics Ever' (**NAE**) birds [15,16] Regulatory amendments such as the Veterinary Feed Directive (**VFD**), have reinforced efforts to find viable alternatives to antibiotics to maintain bird health. Bird health is often compromised in commercial production from recycling litter and subjecting birds to an environment with pathogens. Recycling litter is a common practice in a dual purpose effort to maximize efficiency, and become environmentally conscious. *Eimeria* species and *Clostridium perfringens* are identified as being most problematic to the poultry industry, resulting in huge economic losses caused by coccidiosis and necrotic enteritis respectively.[17,18; 19] Probiotics, often termed Direct Fed Microbial (**DFM**), appear to have benefits when used as a feed additive in poultry production. A single defined mode of action of DFMs has not yet been defined, due to variation of microorganisms used in applications. However, Flint and Garner proposed three mode of actions thus far, chemical inhibition, competitive exclusion, and microbially mediated immunodevelopment [20]. Chemical inhibition is a product of short-chain fatty acids and their ability to either destroy pathogens directly or create "microenvironments" of unfavorable conditions for pathogenic growth. Competitive exclusion suggests that using these microbes as a feed additive allows them to attach themselves to epithelial cells of the gastrointestinal tract, preventing attachment of pathogens. The last mode of action refers to an increase in various immunoglobulin antibodies seen by another group of authors [21].

Although multiple microorganisms have been approved for livestock feed additives, *Bacillus spp.* has become more prevalent because of their ability to form endospores within the gastrointestinal tract, and have increased thermal tolerances to withstand thermal processing such as pelleting. The mode of action that appears to be associated with *Bacillus* spores is increased

immune function. Multiple studies have shown that these spore forming bacteria do have ability to improve performance in both natural and advertently inoculated broilers. [22]

PHOSPHORUS, PHYTATE, and PHYTASE

Phosphorus (**P**) is an important nutrient in commercial broiler diets. Its main role within broiler production is to provide a solid skeletal base that prepares the bird to withstand rigors of rearing, transport, and processing. Additionally, P is a part of several metabolic functions in the avian model [27]. However, a large challenge in meeting this goal is to determine how much P is available to the bird. Common ideologies within practice presume that non-phytate P (**nPP**) is equivalent to that of available P (**avP**)[28]. The amount of P utilized by the bird can vary dependent of not only other nutritional interactions, but source and quantity of P [29].

Environmental concerns have urged commercial poultry nutritionists to maximize efforts to conserve P usage, decreasing both diet costs and excess P presence of litter [30]. Runoff concerns involve litter application post market to field crops, notably within the Mid-Atlantic region. Litter is often high in P, due to an overage of P within diets to meet requirements. Continuing efforts to maximize P digestibility through enzymatic feed additives will assist in the reduction of runoff and subsequent algal blooms.

The main hindrance of P utilization within poultry diets is often related to phytate. Its presence is largely found in plant-based feed stuffs. Poultry are unable to utilize phytate in its common form, phytic acid, due to the lack of effective endogenous enzymes [31]. Phytic acid has the ability to bind minerals and render them unavailable to the bird.

Most diets now mandate an exogenous enzyme known as phytase to aid in release of P and allow chelated minerals such as zinc, magnesium, and calcium to be utilized by the bird.

Phytase enzymes became commercially available in 1991, but have been utilized in research trials dating back to 1962[32]. Modern day phytase additives accommodate a large range of traits for multiple applications. Point of hydrolysis, pH profile and pelleting temperature often place products into respective categories [33]. Point of hydrolysis refers to the location of the carbon (1v4) on the inositol ring that first starts to de-phosphorylate. Fungal derived phytases are the most common source, however recent efforts have been made to implement alternatively derived sources of phytases. Transgenic grain phytases have been used historically in applied research with varied results. **[**34**]** As we continue to understand and develop these phytase products, achieving a more efficient feeding strategy will help prevent over utilization of P in commercial diets.

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CHAPTER 2: Performance of broilers with added direct-fed microbials to a diet in a challenged environment

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SUMMARY Animal health is a key component of rearing commercial chickens for meat production. Previously, antibiotics were fed at sub-therapeutic levels to aid in prevention of disease, most notably coccidiosis. Antibiotic alternatives are essential for the production of poultry within current regulations. Prior research has suggested using *Bacillus spp.*, a direct-fed microbial (DFM), in diets to promote immune function, often resulting in improved bird performance. The study objective was to evaluate untested strains of *Bacillus spp.*, on broiler performance. A basal diet was split equally into 4 allotments, with the difference among treatments being, one control diet (PC), one proven serotype plus PC (DFM1), and two experimental serotypes plus PC (DFM2 and DFM3). All treatments were pelleted and provided in similar feed forms among growth phases. A total of 24 replications per treatment were fed to broilers from d1 to d42. Birds were reared via floor pen in a naturally challenged environment with built-up litter and a diet formulated to have limited nutritional safety margins. Birds fed PC showed marginal increased live weight gain (LWG) as compared to diets containing experimental serotypes ($P \leq 0.05$), for the overall experimental period (d1-42). Feed Conversion Ratio (FCR) showed no differences among treatments. The proven strain of *Bacillus spp.* yielded results comparable to the PC for all growth phases. Individual growth phases yielded no significant differences among the dietary treatments. The experimental *Bacillus spp.* did not improve bird performance relative to a diet without supplementation of a direct-fed microbial.

Key Words: probiotic, direct-fed microbial, antibiotic alternative, *Bacillus subtilis*

DESCRIPTION OF PROBLEM

Antibiotics (**AB**) have historically been utilized to control disease and also aid in growth of commercial poultry produced for human consumption. The United States Food and Drug Administration first approved the use of antibiotic feed additives without veterinary prescription in 1951[1]. Disease control has been connected to feeding AB at sub therapeutic levels to suppress pathogens within the gastrointestinal tract (GI). Consumer and academic scrutiny has led to the abolishment of AB inclusion within animal feeds for food-producing animals. The most concerning topic is antimicrobial resistance in human medicine as a product of AB use in food-producing animals[2]. In 2006, the European Union (EU) placed a ban on sub therapeutic AB use in animal feeds. The United States followed suit a decade later in January of 2017, the veterinary feed directive (VFD) took effect, restricting sub therapeutic AB within feed. Those AB placed on a restricted list are those of medical important to humans. Continued research efforts have been pronounced in finding viable alternatives to antibiotics to minimize economic losses to diseases previously treated or prevented with AB.

An ongoing investigative category of feed additives that are most commonly researched are probiotics. Most probiotics are administered through the feed and are commonly labeled as Direct-Fed Microbials (DFM). The accepted definition is as follows: "Bacteria that have beneficial impacts on their host microbiome and physiology, when fed at adequate amounts." [3] Specific to poultry, prior research has been conducted on *Lactobacillus spp.* and *Bacillus spp.* with success of improving growth performance for both sources of microbials[4-6]. In more recent years, success has been noted of using *Bacillus spp.* in poultry diets as part of a coccidiosis program[7]. *Bacillus* spores of various strains have been promoted for use, due to their ability to withstand not only harsh conditions of the GI, but also during feed manufacture

most notably, pelleting. Spore based probiotics offer more success when multiples strains are utilized, as compared to single strain.

The objective of this study was to evaluate a tested and previously untested serotypes of *Bacillus subtilis* on broiler liver performance, utilizing broilers in a challenged floor-pen environment.

MATERIALS AND METHODS

Experimental diets were formulated to be corn and soybean meal based with an inclusion of corn DDGS and meat and bone meal (Table 1). The diets were in agreement with sponsorship's request to be on the lower end of nutrient densities in commercial practice with limited nutritional safety margins for most nutrients. The addition of a commercial phytase product was consistent among diets, with a suggested P and Ca sparing effect of 0.15 and 0.12%, respectively. [8] Dietary treatments were manufactured at the West Virginia University Pilot Feed Mill in Morgantown, West Virginia, using a 40 HP California Pellet Mill. [9] A premix of micro-ingredients was made for each diet according to formulation. A basal diet was mixed via a one-ton vertical screw mixer. [10] Basal diet was replicated and randomly allotted to 4 treatments to assimilate nutrient content. On day of manufacture, *Bacillus spp*. products[8, 11], were added to a 3kg sample of basal feed in a Univex Mixer [12], and mixed for 5 minutes before being remixed with allotted feed in the vertical screw mixer. Inclusions of each product were determined by manufacturer's recommendation to achieve target CFU/kg of feed. All dietary fat was added at the mixer. All four treatments per growth phase (starter, grower, and finisher) were pelleted on the same respective day. Diets were conditioned at 76.6℃ for 15 seconds utilizing a California Pellet Mill Conditioner (457 x 1981 mm) and then passed through the barrel of a Hygienizer for 45 seconds. Feed is required to pass through the Hygienizer before being extruded; it should be noted that the steam jacket for the Hygienizer was off during this study. Feed was then extruded through a 4.8 x 38.1 mm pellet die. Hot pellet samples were collected immediately following pellet extrusion through the pellet die and used to measure hot pellet temperature. Samples were also collected from the pellet die and placed on a large fan to be cooled and subsequently analyzed for nutrient content- Feed samples were sent to a

commercial laboratory [13] for analysis of ,crude protein, crude fat, ash, total phosphorus, phytic acid, and calcium content of finished feed. Total phosphorus and phytic acid analyses were used to calculate nPP [14].

The sampling method followed Reese et. al., [15] for proper diet validation. Starter diets (d1-14) were crumbled so that chicks could easily consume the feed on D1. Particle size of finished feed was manipulated by changing the gap distance between rolls on the roller mill. Grower (d15-28) and Finisher (d29-42) were fed as intact pellets. Feed samples were collected from the cooler deck for analysis of pellet durability. Particle size analysis was conducted on the starter diet, while percent pellet analysis was conducted on grower and finisher diets. Pellet durability analyses were conducted on all treatments for all three growth phases. Pellet durability was analyzed using three separate methods that varied in mechanics. Pellet Durability Index (PDI) and modified Pellet Durability Index (MPDI) were determined using a Pfost tumbler box [16]. New Holmen Pellet Tester (NHPT) was utilized 24h post pelleting [17]. Particle size for starter complete feed was determined using a 100g sample passed through a sieve shaker for 10 minutes [18]. Percent pellet analysis for grower and finisher diets were determined by placing 22.7kg of complete feed on a ASAE #5 sieve and calculated as the percent of pellets remaining after sieving process [19]. Descriptive feed manufacture data can be found in Table 2.

Live Birds and Housing

A total of 2,208 Hubbard x Ross 708 male day old broilers chicks were obtained from a commercial hatchery [20] and vaccinated for Marek's, New Castle, and coccidiosis. Chicks were weighed, sorted, and allocated to have similar starting pen weight by block. Birds were placed at a count of 23 broilers per pen in 96 pens (0.69 X 2.44 m). Pens were divided between 3 rooms joined together with woven wire, allowing heat and ventilation between all three. Rooms are

located in a cross-ventilated negative pressure barn. Litter was built up from one previous flock and sprayed once per week with a light water spray to facilitate bacterial growth. Previously mentioned methods created a natural challenge for birds. The four dietary treatments were randomly allotted to adjacent pens blocked by location in the rooms located at the West Virginia University Animal Sciences Farm. Each dietary treatment was applied to 24 replicate pens of broilers. Lighting was continuous from d1-3, reduced 1 hour per day from d4-7, reduced 4 hours per day from d8-14 and reduced 6 hours per day from d15-42 based off guidelines provided by a commercial rearing handbook [21]. Feed and water were provided ad libitum throughout the study and temperature was manipulated daily based on the Aviagen Broiler Handbook. [22] Initial starter feed provided on trays within each pen until d7, afterwards feed pans with attached hoppers would be used for the remainder of study. [23] Nipple drinkers provided water to approximately 12 birds/nipple [24]. Mortalities were replaced from d1 to d3.

Variables measured included feed intake (FI), bird live weight gain (LWG), mortality corrected feed conversion ratio (FCR), and percent mortality (Mort). At the end of the study (d42), birds were individually weighed to calculate coefficient of variation (CV) to view weight uniformity among pens. Additionally, 3 broilers per pen (±100g of mean pen weight) were selected and euthanized via cervical dislocation for cecal collection. All animals were reared according to protocols established by the West Virginia University Animal Care and Use Committee, protocol #1602000612.

Statistical Analysis

Performance variables were analyzed using a randomized complete block design. The experimental unit was 1 pen of 23 broilers. Data were analyzed using the PROC GLM method of Statistical Analysis System[24] for a one-way ANOVA with alpha designated at 0.05. When

means were revealed to be significantly different, means were separated using Fisher's LSD post hoc comparison.

RESULTS AND DISCUSSION

Data from feed manufacture and live performance are in Tables 2 and 3, respectively. Feed manufacture and pellet quality measures were not replicated and should be interpreted as descriptive data. Pellet durability index (PDI) ranged from 44.92 to 49.51%, 36.98 to 49.90%, and 34.10 to 45.29% respectively for starter, grower, and finisher periods. These values were expected from the manufacturing technique of applying all dietary fat at the mixer, decreasing time of conditioned feed within the pellet die. High inclusions of mixer-added fat (MAF) have been shown to decrease pellet quality, but aid against detriments to nutrient availability associated with pelleting [25]. Starter particle size ranged from 975 to 1263 microns. Grower and finisher percent pellet ranged from 34 to 44% and 23 to 35%, respectively. These values corresponded with pellet quality data, utilizing a larger representative sample of feed. Nutrient analyses of the complete diets best mimicked calculated nutrients during the finisher phase. Starter and grower phase analyses yielded crude protein values lower than expected. Source of soybean meal was thought to explain why these values were lower than calculated values.

Live Performance.

Measures of LWG, FI, FCR and mortality are shown in Table 3. Pen starting weight were not different by design with an average of 1.05 kg per pen ($P = 0.6095$). Creating the natural challenge is necessary to hinder growth of control diet, promoting efficacy of microbial additives to manage environmental stressors. A natural challenge appeared to be present, as performance metrics were less than industry guidelines for this broiler strain [26]. Although birds fed the PC treatment did not meet industry standards for LWG, a significant increase ($P=0.549$) could be seen relative to both treatments containing experimental DFMs. This difference in LWG was marginal and did not establish differences for FCR. Numerous studies indicate improvements of

LWG with broilers fed diets containing DFM [26-28]. These experiments that generated positive results in LWG used *Bacillus*-based DFM. No significant differences in FI were observed among treatments (Table 3). Average FI (kg per bird) across treatments were 0.432 (P = 0.61), 1.444 (P = 0.94), and 2.152 (P = 0.49) for the 1-14, 15-28, and 29-42 d periods. Notable differences among FI have been observed from other studies, notably studies that induce challenge through oral gavage around d 19 to 21. Variation of both increasing [27], and decreasing FI [29,30] are thought to vary partially by date of pathogen introduction and also combination effects albeit, diet formulation or DFM with added enzyme cocktails (Xylanase, Phytase, Amylase, etc.). Overall FCR differences were not observed in the current study (P = 0.84). Conversely, Tactacan et al., found that a *Bacillus subtilis* derived DFM can yield similar FCR to that of a diet containing an antibiotic growth promotor, while subjected to a Necrotic Enteritis challenge [31]. In the same study, it was observed that a 1-log reduction in colony count of DFM would not exhibit the same improvements to FCR during the challenge, indicating adequate amounts of DFM must be supplied. The performance results of this study indicate that the natural challenge may have been too large for additives to overcome a multitude of environmental stressors.

CONCLUSIONS AND APPLICATIONS

1. The natural challenge in this study could not be overcome by experimental DFMs.
2. The two experimental microbes (DFM 2 and 3) decreased performance.
3. DFM1 showed similar performance as a diet without any microbial product.

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before being deposited into a Pfost tumbler. The sifted pellets were then tumbled in the container, dimensions 5 × 12 × 12 in., with a 2 × 9 in. plate fixed diagonally along the 12 × 12 in. side, for approximately 10 min at 50 rpm. The sample was then sifted again through the No. 5 (ASTM) mm screen, weighed, and the percentage of pellets was calculated by dividing the weight of pellets after tumbling by the weight of pellets before tumbling and then multiplying that value by 100. Modified pellet durability index was similarly measured, with the exception of the addition of five, 13-mm hexagonal bolts to the 500 g sample in the tumbler. Both analyses are meant to simulate the deleterious effects of transferring and handling the pellets, 1983

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Table 1. Ingredient and nutrient composition of diets through each growth period.

	Positive Control		
Ingredients (%)	**Starter**	**Grower**	**Finisher**
Corn	64.93	62.63	61.46
Soybean Meal (48%)	24.14	25.49	27.44
Porcine Meat and Bone Meal	4.00	2.99	1.98
Soybean Oil	2.22	3.45	4.26
Corn DDGS	1.76	3.00	2.66
Limestone	1.00	0.88	0.94
Methionine	0.49	0.41	0.35
Lysine	0.39	0.20	0.03
DiCalcium Phosphate	0.26	0.20	0.20
Poultry Vitamin Premix[5]	0.25	0.25	0.25
Salt	0.23	0.26	0.29
Threonine	0.19	0.10	0.009
Sodium Bicarbonate	0.10	0.10	0.10
HiPhos 2500 GT[4]	0.04	0.04	0.04
Calculated Nutrients (%)			
ME (kcal/kg)	3020	3108	3164
Crude Protein	19.00	19.00	19.00
Dig Lysine	1.15	1.02	0.92
Dig SAA	1.00	0.93	0.89
Dig Valine	0.87	0.88	0.91
Dig Threonine	0.77	0.69	0.62
Dig Methionine	0.76	0.69	0.63
Available Phosphorus	0.33	0.28	0.24
Sodium	0.17	0.18	0.18
Calcium	0.88	0.73	0.66

[1]Diets were formulated to be on the lower end of commercial diets with limited safety margins for all nutrients.
[2]Direct-fed microbial (*Bacillus subtilis*) produced by Chr Hansen (Hoersholm, Danmark)
[3]Experimental *Bacillus subtilis* products, DSM Nutritional Products Inc. (Parsippany, NJ)
[4]Commercial phytase to replace 0.15% P & 0.12% Ca. (DSM Nutritional Products, Inc., Parsippany, NJ)
[5]Supplied the following per kilogram of diet: manganese, 0.02%; zinc, 0.02%; iron, 0.01%; copper, 0.0025%; iodine, 0.0003%; selenium, 0.00003%; folic acid, 0.69 mg; choline, 386 mg; riboflavin, 6.61 mg; biotin, 0.03 mg; vitamin B6, 1.38 mg; niacin, 27.56 mg; pantothenic acid, 6.61 mg; thiamine, 2.20 mg; menadione, 0.83 mg; vitamin B12, 0.01 mg; vitamin E, 16.53 IU; vitamin D3, 2,133 ICU; vitamin A, 7,716 IU.

Table 2. Analyzed Nutrients (%)

Treatment	Growth Period	Crude Protein[1]	Crude Fat[2]	Ash[3]	Calcium[4]	Total Phosphorus[5]	nPP[6]
Control	Starter	17.3	5.45	4.39	0.91	0.53	0.34
	Grower	17.9	6.32	3.97	0.70	0.49	0.29
	Finisher	19.5	7.42	4.10	0.67	0.47	0.26
Control + DFM1 (0.10%)	Starter	17.6	5.70	4.39	0.98	0.53	0.33
	Grower	17.4	6.59	3.85	0.74	0.47	0.26
	Finisher	19.0	7.74	3.99	0.69	0.47	0.24
Control + DFM2 (0.32%)	Starter	17.6	5.42	4.79	0.91	0.53	0.32
	Grower	18.0	6.61	4.12	0.82	0.49	0.26
	Finisher	18.2	7.49	4.21	0.76	0.46	0.24
Control + DFM3 (0.32%)	Starter	18.4	5.70	4.53	0.95	0.52	0.34
	Grower	18.7	6.21	4.13	0.77	0.49	0.28
	Finisher	18.8	7.62	4.26	0.69	0.45	0.22

[1]AOAC 992.15, AOAC 990.03, AOCS Ba 4e-93; Combustion.
[2]AOAC 920.39; Ethyl ether extraction.
[3]AOAC 923.03
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[6]Non-phytate phosphorus = total phosphorus (AOAC 965.17/985.01 mod) – [0.282 X phytic acid (Analytical Biochemistry Vol. 77:536-539, 1977)] x 100.

Table 3. Descriptive Feed Manufacture Data for Starter, Grower, and Finisher Periods.

Treatment	Growth Period	Manufacture Data			Pellet Durability Data			Particle Analysis	
		Mill Load[1] (%)	Hygienizer Temp[2] (^{0}C)	Hot Pellet Temp[3] (^{0}C)	NHPT[4] (%)	PDI[5] (%)	MPDI[6] (%)	Crumble Particle Size[7] (µm)	Percent Pellet[8] (%)
Control	Starter	42	58.3	76.6	21.80	49.51	36.57	1263 ± 2.0	-
	Grower	41	52.8	74.3	26.20	49.90	35.01	-	34.47
	Finisher	40	61.7	73.0	18.71	34.10	21.01	-	35.14
Control + DFM1 (*.10%)	Starter	42	61.1	76.7	18.60	47.89	35.86	1088 ± 2.0	-
	Grower	42	60.0	73.5	16.74	36.98	23.12	-	34.00
	Finisher	41	60.0	72.6	35.50	45.29	33.07	-	27.90
Control + DFM2 (*.32%)	Starter	42	62.2	77.3	19.34	45.67	32.22	1218 ± 2.0	-
	Grower	42	61.1	74.2	24.97	46.48	32.45	-	40.52
	Finisher	41	60.6	72.4	19.24	35.30	21.65	-	25.34
Control + DFM3 (*.32%)	Starter	42	63.9	77.7	19.23	44.92	33.99	975 ± 2.0	-
	Grower	43	61.7	72.8	18.50	41.09	25.47	-	43.92
	Finisher	41	60.0	73.2	25.38	40.44	19.58	-	23.20

[1]A 100% motor load was based on FLA (full load amps) based on the pellet mill motor name plate.

[2]The hygienizer was not turned on during this experiment; however, feed must run through the hygienizer for 45 seconds post conditioning and prior to pellet die extrusion based on the WVU feed manufacture system.

[3]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.

[4]New Holmen Pellet Tester is run using 100g of sifted pelleted samples that are subjected to air flow within a perforated chamber for 30s.

[5]Pellet durability index was determined by placing 500g of sifted pellets into a Pfost tumbler. Samples were tumbled for 10 min at 50 rpm. The sample was then sifted again and weighed. Pellet durability index was calculated as the percentage of sifted pellets retained after tumbling.

[6]Modified pellet durability index was measured similarly to the previous description, with the exception that five 13-mm hexagonal nuts were added to the 500-g sample before tumbling.

[7]100 g of crumbled diets placed within WS Tyler Ro-Tap Sieve Shaker and run for 10 minutes, contents of each sieve was weighed back to determine particle size.

[8]22.7 kg of complete feed is passed through a No. 5 Tyler Sieve. Pellets remaining on sieve are weighed back and calculated as a pellet percentage.

*Manufacture data is recorded when PLC(Programmable Logic Control) indicates the conditioner is at desired temperature (76.6^{0}C).

**Diets were manufactured with 275.8 kPa prior to Mason-Neilan valve.

16 # Table 4. Performance Data for Individual Growth Periods

Treatment	D1-14					D15-28					D29-42				
	Starting Bird Wt (kg)	**Feed Intake / Bird (kg)**	**LWG[1] / Bird (kg)**	**FCR[2]**	**Percent Mortality (%)**	**Starting Bird Wt (kg)**	**Feed Intake / Bird (kg)**	**LWG[1] / Bird (kg)**	**FCR[2]**	**Percent Mortality (%)**	**Starting Bird Wt (kg)**	**Feed Intake / Bird (kg)**	**LWG[1] / Bird (kg)**	**FCR[2]**	**Percent Mortality (%)**
Control	0.0458	0.434	0.317	1.37	2.36	0.363	1.450	0.887	1.64	0.55	1.250	2.194	1.060	2.07	2.26
Control + DFM1 (*.10%)	0.0458	0.432	0.317	1.36	1.99	0.362	1.444	0.884	1.64	0.93	1.247	2.147	1.043	2.07	0.36
Control + DFM2 (*.32%)	0.0457	0.432	0.315	1.37	1.81	0.361	1.443	0.882	1.64	1.49	1.243	2.147	1.020	2.12	0.76
Control + DFM3 (*.32%)	0.0459	0.431	0.313	1.37	0.91	0.359	1.439	0.871	1.66	0.54	1.230	2.120	1.016	2.08	1.65
P-value	0.6095	0.9499	0.7810	0.8710	0.2151	0.7972	0.9376	0.3469	0.5381	0.4978	0.3266	0.4922	0.4586	0.7255	0.1042
SEM[4]	0.00005	0.004	0.003	0.011	0.4989	0.008	0.012	0.007	0.012	0.4983	0.008	0.034	0.022	0.033	0.5889

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Table 5. Performance Data for Overall Growth Period

Treatment	D1-42			
	Feed Intake / Bird (kg)	**LWG[1] / Bird (kg)**	**FCR[2]**	**Percent Mortality (%)**
Control	4.078	2.291[a]	1.78	5.53
Control + DFM1 (*.10%)	4.013	2.242[ab]	1.79	3.16
Control + DFM2 (*.32%)	3.979	2.186[b]	1.82	4.35
Control + DFM3 (*.32%)	3.945	2.204[b]	1.79	2.96
Treatment *P*-value	0.3942	**0.0549**	0.8445	0.0875
Treatment SEM	0.047	0.023	0.016	0.8755
Ross Guidelines For D42	**4.533**	**2.840**	**1.649**	-

[a-b]Means within the same column with no common superscript differ significantly ($p \leq 0.05$)
[1]Live weight gain
[2]Feed conversion ratio

FORMATTED FOR PUBLICATION IN THE JOURNAL OF APPLIED POULTRY RESEARCH

CHAPTER 3: Effects of phytase activity concentration in grain affects mixer homogeneity, broiler performance, and tibia mineralization in male broilers

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SUMMARY This study hypothesized that phytase activity concentration in grain affects mixer homogeneity, thermal stability post pelleting, and efficacy in regard to growth performance and tibia mineralization of broiler chickens. The objective of the study was to feed broilers two corn-expressed phytase products that differed in activity concentration (CEP1: 3,000 FTU/g and CEP2: 12,036 FTU/g) and three doses (3,000, 6,000, and 9,000 FTU/kg). Differing experimental product and target dose varied volume of enzyme addition to evaluate mixer homogeneity, broiler performance, and tibia mineralization. Dietary treatments included a positive (PC) and negative control (NC) (0.15% decrease in Ca and nPP compared to PC), and six additional diets containing CEP products within the NC formulation at each target dose. Birds were fed a starter (d1-10), grower (d11-21), and finisher (d22-42) diet. All diets were conditioned at 75˚C for 15s and fed to 12 replications of 24 male Hubbard x Ross 708 broilers housed on floor pens. Treatments were arranged in a 2 (concentration) x 3 (dose) factorial in a randomized complete block design. Broilers provided PC and NC produced expected performance and tibia mineralization differences. Mixer CV based on phytase activity resulted in lower values for CEP1 compared to CEP2 for the starter phase. A concentration x dose interaction occurred for d10 FCR ($P < 0.05$). Day 10 FCR increased as inclusion rate increased for CEP2, and FCR decreased as dose increased from 3,000 to 9,000 FTU/kg for CEP1 ($P = 0.0275$). During the starter phase, birds fed diets with CEP1 consumed more feed than those fed CEP2 ($P<0.05$). As dose increased, grower phase LWG also increased ($P<0.05$). On d42, ending bird weight and subsequent LWG increased when birds were provided CEP1 compared to CEP2 ($P < 0.05$). Tibia ash was highest at 6,000 and 9,000 FTU/kg on d21 and lowest at 3,000 FTU/kg ($P < 0.05$). On d42, birds provided CEP1 had a higher tibia ash per bird compared to birds provided CEP2 ($P = 0.0280$). These data suggest that a corn-expressed phytase product with a low concentration may

provide better broiler performance and tibia mineralization compared to a phytase product with higher concentration, likely caused by a more uniform mix.

Key Words: concentration, inclusion level, corn-expressed phytase, tibia mineralization, mixer homogeneity

DESCRIPTION OF PROBLEM

Commercial broiler diets are formulated through a systematic approach to meet nutrient requirements, while also limiting diet cost. Formulating on digestible nutrient values has demonstrated increased utilization of nutrients per respective ingredient[1,2]. Exogenous enzymes have allowed further improvements regarding performance [3,4]. One such enzyme, phytase, has been added to diets to improve Phosphorus (P) digestibility of plant-based feedstuffs, and reduce P excretion in the litter [5].

Historically, these enzymes are of a microbial source and generally lack thermal stability unless coated [6]. Transgenic grain enzyme technology has been effective at expressing phytase in animal feeds that result in similar or improved performance compared to microbial/fungal sources[7,8]. It also has been shown that corn-expressed enzymes are able to reduce anti-nutritional properties associated with non-starch polysaccharides [9]. Grain-expressed enzymes may offer added practical advantage during diet formulation. The grain itself being composed of a specific enzyme allow for practically zero nutrient dilution unlike microbial derived enzymes. Uniform mix is important during feed manufacture to distribute all ingredients adequately. Subsequently, nutrient densities will be similar within a properly mixed formulation. Equal nutrient densities will supply the animal with a balanced diet, maximizing performance [10]. Most broiler diets have a variety of low-inclusion ingredients such as crystalline amino acids, vitamin and mineral premixes, and exogenous enzymes. Pelleted feed has been shown to improve performance and provide economic benefit despite requiring additional energy to manufacture [11]. These improvements may not be apparent if proper mixing was not achieved. Although there are differences of phytase origin, these phytases also differ in varying concentration between source of manufacture. This creates a discrepancy of volume required to

achieve target dose between two products. Throughput of commercial mills is often elevated in order to meet production quotas, while still producing feed of equal nutritional density. Therefore differing concentrations of dietary enzymes needs to be evaluated as a method of determining mixing homogeneity and its translation to performance.

The current study investigated the effects of feeding two different grain-expressed phytase products at three increasing doses and its effect on mixer uniformity, broiler performance, and bone mineralization.

MATERIALS AND METHODS

Experimental Design

Differences in phytase activity of transgenic corn were evaluated within a randomized complete block design. Treatments were arranged as two corn-expressed phytases differing in expression rates, at three target doses, along with a positive and negative control (reduced 0.15% in Ca and nPP), for a total of 8 treatments. The three target levels of phytase, 3,000, 6,000, and 9,000 FTU/kg, were obtained using different loading rates for both the Corn-Expressed Phytase products (CEP1 and CEP2) [Table 1, 12]. The difference in expression rates generated different volumes of each respective phytase product (CEP1: 3,000 u/g and CEP2: 12,036 u/g). Phytase analysis for 10 samples per treatment were analyzed to determine coefficient of variation to assess distribution within the mixed batch of feed (Table 2).

Experimental Diets

Experimental diets were corn and soybean meal based. In table 1, the PC and NC diets are shown. The remaining diets were generated with addition of product at target dose. Phytase product is expressed within the corn grain and was included in treatments at the expense of corn (1:1). Diets were manufactured at the West Virginia University pilot feed mill using a 40 HP California Pellet Mill [13]. Prior to pelleting, basal batches of diets were weighed, mixed, and allotted to all 8 treatments. A premix including the phytase had been mixed in a Univex mixer for 5 minutes, along with 3kg of basal diet before being remixed on day of manufacture [14,15]. Diets were conditioned, pelleted, and bagged to be fed to broilers [16]. Hot pellet samples were collected immediately following pellet extrusion and placed on a large agricultural fan to be cooled for nutrient analysis following methods described by Reese et al [17]. Starter (d1 to 10) and grower (d11 to 21) diets were passed through a roller mill to generate a crumble form for

each period. Particle size of finished feed was manipulated by changing the gap distance between rolls. Starter diets were determined to be a fine crumble, whereas grower feed, a coarsely crumbled diet to prepare broilers for intact pellets in the finisher (d22 to 42) phase. Descriptive feed manufacture data can be found in Table 2, describing pellet durability, pelleting temperature, and particle size of treatments for each phase.

Live Birds and Housing

A total of 2304 Hubbard x Ross 708 male broiler chicks were obtained from a commercial hatchery on day of hatch [18]. Birds were vaccinated for Marek's, New Castle and coccidiosis. Twenty-four birds were randomly allotted to 96 floor pens containing a plastic feed tray and nipple drinker. Pens contained built-up litter from two previous flocks and were top dressed with fresh pine shavings. Temperature and lighting followed the Ross Broiler Management Handbook [19]. Variables measured included feed intake (FI), bird live weight gain (LWG), mortality corrected feed conversion ratio (FCR), and percent mortality. On d 21 chicks were weighed as a pen and 5 chicks per pen were randomly selected and euthanized for tibia excision and ash analysis [20]. Similar methods were used at the end of the study (d42), with three birds being selected for tibia excision.

Statistical Analysis

Overall comparisons were analyzed as a one-way ANOVA including all treatments in a randomized complete block design. A factorial analysis was performed on 6 treatments of factorial structure, disregarding controls. Main effects were considered as product concentration and inclusion level or dose. Main effect interactions were also considered. Means were further explored using Fisher's Protected LSD test when main effect interactions were significant at the $P \leq 0.05$. One pen of broilers was defined as the experimental unit. Blocking was based on pen

location within the research barn. Experimental period were 42 days, segmented into three growth phases, starter, grower, and finisher. Data was analyzed for each growth period as well as total study period using the PROC GLM method of SAS [21].

RESULTS AND DISCUSSION

Feed Manufacture

Feed manufacturing and pellet quality data were not replicated and should be considered as descriptive (Table 2). Pellet durability index (PDI) values ranged from 61.5% to 74.9%, 51.6% to 58.5%, and 58.3% to 72.3% for starter, grower, and finish phases respectively. An overall decreased quality range for the grower phase is likely associated with the ambient temperature of -4^0C during manufacture. Positive control treatment appeared to have higher quality for PDI; this was also observed for both modified pellet durability and New Holmen pellet tester (not shown). The PDI best represents feed quality in current controlled research setting as feed is minimally handled. Incremental increases of particle size could also be seen respective of growth phase to prepare broilers for in-tact pellets. Mineral analysis (Table 4) post manufacture, indicated reduced levels of non-phytate Phosphorus (nPP) and Calcium (Ca) in all treatments with negative control formulation base. This was expected in place of the sparing matrix values of the phytase used in this study.

Phytase Analysis

Phytase analysis data can be seen in Table 3. Starter phase analysis for all treatments was performed on 10 samples of each diet. Within each of the 10 samples, multiple analysis was performed. As inclusion level increased, CV decreased for both phytase supplements. The CEP1 phytase grain consistently produced a CV value of less than 10, often thought to be an industry standard. This would confirm the hypothesis that more volume has higher success of uniform distribution in a batch of feed. This is further supported that as inclusion level of each respective product increased, CV also decreases. Analysis did suggest that the middle inclusion level may have not been achieved in mash diets, but studies have suggested phytase analysis is variable [22]. Furthermore, starter pellet samples indicated approximately 50% retention of phytase

activity, post thermal pelleting. The levels fed did however remain in stepwise order from lowest to highest. Analysis of CEP2 for pellet did yield erratic results in attempting to meet target activities. Phytase activity can be considered as a "superdose" (>1500 FTU/kg) for all inclusion levels. A super dose has been found to alleviate gastrointestinal irritation caused by phytate molecules [23,24]. Expected increases in performance are expected for phytase containing treatments.

Dietary Phase Bird Performance

Performance data were analyzed in overall comparison with control diets -and with only factorial treatment structure to determine interaction or main effect differences. Table 5 shows performance data for each phase. Within the starter period, birds fed a NC diet without grain phytase had decreased LWG across all treatments (P=0.0290). Additionally, birds consuming diets containing CEP1 consumed more feed than those fed CEP2 containing diets (P=0.0298). Differences were not observed in FCR. Decreased body weight could have been caused by less bone mineralization from a Ca and P deficient diet. In the factorial analysis of phytase containing diets, an interaction was observed for FCR (P=0.0275). In Figure 1, the CEP2 phytase yielded a lower FCR marginally for the 3,000 and 6,000 inclusion levels, but increases at highest dose (9,000 FTU/kg) for this study. This supports the theory that a larger volume of enzyme can be more adequately distributed within a batch of feed provided to birds. Grower phase results are similar with respect to birds fed NC diet had decreased LWG than treatments including phytase or PC (P<0.0001). Additionally, FI was also significantly decreased (P<0.0001). Increasing dose of phytase activity increased LWG in phytase containing diets (P=0.0330). No interactions were apparent, unlike starter phase. Finisher phase contrasted results of initial two phases for performance, indicating only trends for LWG and FI in overall comparison (P=0.08). Factorial

analysis revealed a significant main effect for product as improvements to live weight gain for birds that consumed the CEP1 phytase that requires more volume (P=0.0328).

Overall Period (d1-42) Performance

Overall performance was similarly analyzed in an overall multiple comparison with control diets and the factorial treatment structure was analyzed separately (Table 6). Diets containing phytase appeared to spare Ca and P to yield similar ending bird weights relative to birds fed a diet with adequate Ca and P (P=0.0063). The birds fed the same diets (all but NC) also consumed more feed than the NC diet (P=0.0257). Main effect for product resulted in larger birds or increased LWG for birds fed diets containing CEP1 versus CEP2. The overall difference was approximately 70g (P=0.0352). No interactions were seen for overall performance. FCR also was not significant for full experimental period (P>0.05).

Tibia Mineralization

The tibia mineralization results are presented in Table 7 in two expressions. Total mg of ash and percent ash of total tibia weight were analyzed. Significance was observed at the end of both d21 and d42. Diets containing phytase differed in tibia ash percentage and mg tibia ash per chick on d21 with lowest dose yielding lower values relative to both increased doses (P=0.0024 and 0.0002). Following the finisher phase, the results differed. Tibia ash percentage was effectively the same disregarding the NC diet. Main effect was lost for dose, but the product main effect appeared in mg tibia ash per chick. The birds fed CEP1 had increased levels of tibia ash on a per chick basis compared t those fed diets containing CEP2. These data support the overall performance main effect that CEP1 improves LWG but also improves tibia mineralization. A lower concentrated product may be better distributed throughout batches of feed.

CONCLUSIONS AND APPLICATIONS

1. Grain-expressed phytase products can provide Ca/P sparing effects in corn-soybean meal based diets.
2. Increasing inclusion level of phytase will result in an improved distribution within a batch of feed, ultimately translating to performance.
3. Grain-phytase products expressed with lower concentrations(~3000 u/g) result in improved LWG over the life of the bird in this study because of improved distribution in feed.

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Table 1. Diet composition of positive and negative control diets[1] for starter, grower, and finisher periods)

Ingredient	Starter(D1-10)		Grower(D11-21)		Finisher(D22-42)	
	Positive Control	Negative Control	Positive Control	Negative Control	Positive Control	Negative Control
	%		%		%	
Corn	54.12	54.83	60.02	60.73	62.40	63.11
Soybean Meal (46%)	38.02	38.02	32.14	32.14	30.14	30.14
Soybean Oil	3.47	3.47	3.76	3.76	3.99	3.99
Limestone	1.07	1.17	1.03	1.13	0.96	1.06
DL – Methionine	0.49	0.49	0.37	0.37	0.24	0.24
Dicalcium Phosphate	1.73	0.92	1.61	0.80	1.40	0.59
Salt	0.33	0.33	0.35	0.35	0.35	0.35
Vitamin Mineral Premix[2]	0.25	0.25	0.25	0.25	0.25	0.25
Sodium Bicarbonate	0.10	0.10	0.10	0.10	0.10	0.10
Lysine	0.12	0.12	0.17	0.17	0.11	0.11
Threonine	0.29	0.29	0.20	0.20	0.06	0.06
Calculated Nutrients						
ME[3] (kcal/kg)	1361	1361	1394	1394	1416	1416
Crude Protein (%)	22.4	22.4	20.0	19.8	18.7	18.7
Digestible Lysine[4] (%)	1.20	1.20	1.10	1.10	1.00	1.00
Digestible Methionine[4] (%)	0.79	0.79	0.65	0.65	0.51	0.51
Digestible Met + Cys[4] (%)	1.09	1.09	0.92	0.92	0.77	0.77
Digestible Threonine[4] (%)	1.01	1.01	0.84	0.84	0.68	0.68
Digestible Tryptophan[4] (%)	0.24	0.24	0.21	0.21	0.20	0.20
Calcium (%)	0.90	0.75	0.84	0.69	0.76	0.61
nPP (%)	0.45	0.30	0.42	0.27	0.38	0.23

[1]Calculated inclusions of experimental corn-expressed phytase was added to the negative control at a 1:1 replacement of ground corn

[2]Supplied per kilogram of diet: 0.02% manganese; 0.02% zinc, 0.01% iron; 0.0025% copper; 0.0003% iodine; 0.0003% selenium; 0.69 mg of folic acid; 386 mg of choline; 6.61 mg of riboflavin; 0.03 mg of biotin; 1.38 mg of vitamin B6; 27.56 mg of niacin; 6.61 mg of pantothenic acid; 2.20 mg of thiamine; 0.83 mg of menadione; 0.01 mg of vitamin B12; 16.53 IU of vitamin E; 2,133 IU of vitamin D3; and 7,716 IU of vitamin A.

[3]Metabolisable Energy and Available Phosphorus were based on Agristat values as suggested by M. Donohue. 2013 [25] Available Phosphorus in the NC were reduced by 0.15.

[4]Digestible amino acids were based on the digestible lysine values suggested by P. B. Tillman and W. A. Dozier. 2013. [26] Digestible amino acid to digestible lysine ratios followed further recommendations in this publication.

[5]nPP = non-phytate phosphorus

Table 2. Phytase Activity in Starter Mash Diets

Treatment		Average Activity[1] (FTU/kg)	Standard Deviation[2] (FTU/kg)	Coefficient of Variation[3] (%)
Product	**Dose**			
CEP1	3,000	2910	249	8.55
	6,000	4290	276	6.44
	9,000	7981	577	7.23
CEP2	3,000	2524	418	16.57
	6,000	5370	747	13.91
	9,000	8499	792	9.32
Negative Control[4]		54	61	-
Positive Control[4]		60	129	-

[1]Ten representative samples were analyzed for phytase activity; each sample was tested eight times.
[2]Standard deviation shows the average standard deviation from multiple analysis of each sample bag.
[3]Coefficient of variation was determined by dividing Standard Deviation by Average Analysis and multiplied by 100 to generate a percentage.
[4]Both control values were generated from one representative sample as they were not treatments of interest, nor were they suspected to contain adequate phytase activity.

Table 3. Descriptive Feed Manufacturing Data for Starter, Grower, and Finisher Growth Periods

Treatment		Growth Period												
		Starter(D1-10)					Grower(D11-21)					Finisher(D22-42)			
Product	Dose (FTU/kg)	Hot Pellet Temperature[1] (^{0}C)	PDI[2] (%)	Particle Size[3](µm)	Phytase Activity[4] (FTU/kg)		Hot Pellet Temperature[1] (^{0}C)	PDI[2] (%)	Particle Size[3] (µm)	Phytase Activity[4] (FTU/kg)		Hot Pellet Temperature[1] (^{0}C)	PDI[2] (%)	Particle Size[3] (µm)	Phytase Activity[4] (FTU/kg)
CEP 1	3,000	73.0	65.58	1101	1500		73.5	54.24	1517	1200		76.8	68.15	3177	3000
	6,000	74.5	65.01	1365	2800		74.1	55.93	1562	4200		74.8	68.00	3522	2900
	9,000	74.4	62.33	1214	3600		72.8	54.38	1580	2800		76.7	62.27	3410	4200
CEP 2	3,000	74.1	61.51	1252	2600		71.8	53.44	1507	530		75.7	60.60	3238	480
	6,000	73.9	62.25	1092	3400		71.7	51.97	1465	5300		74.7	58.31	3360	1700
	9,000	72.9	61.71	1244	3100		72.9	51.58	1491	6100		75.7	61.87	3501	6600
Negative Control		73.4	64.12	1152	170		73.8	52.9	1505	94		75.7	69.10	3104	<70
Positive Control		73.3	74.85	1195	200		71.0	58.50	1692	140		75.9	72.29	3373	<70

[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[27].

[2] Pellet durability index was determined by placing 500g of sifted pellets into a Pfost tumbler. Samples were tumbled for 10 min at 50 rpm. The sample was then sifted again and weighed. Pellet durability index was calculated as the percentage of sifted pellets retained after tumbling.

[3]100 g of complete diets placed within WS Tyler Ro-Tap Sieve Shaker and run for 10 minutes, contents of each sieve was weighed back to determine particle size.

[4] Pelleted samples were collected directly post-extrusion and placed on a fan to cool before analysis using AOAC 2000.12 method.

Table 4. Mineral Analysis and nPP Calculations for Starter, Grower, and Finisher Periods

Treatment		Growth Period											
		Starter (D1-10)				Grower (D11-21)				Finisher (D22-42)			
Product	Dose (FTU/kg)	Total Phosphorus (%)	Phytic Acid (%)	nPP[1] (%)	Calcium (%)	Total Phosphorus (%)	Phytic Acid (%)	nPP[1] (%)	Calcium (%)	Total Phosphorus (%)	Phytic Acid (%)	nPP[1] (%)	Calcium (%)
CEP1	3,000	0.557	0.786	0.34	0.749	0.453	1.06	0.16	0.696	0.438	0.812	0.21	0.706
	6,000	0.541	0.751	0.33	0.752	0.452	0.801	0.23	0.630	0.417	0.760	0.20	0.566
	9,000	0.541	0.877	0.29	0.729	0.485	0.831	0.25	0.749	0.397	0.781	0.18	0.594
CEP2	3,000	0.526	0.843	0.29	0.744	0.500	0.923	0.24	0.693	0.382	0.789	0.16	0.590
	6,000	0.514	0.802	0.29	0.778	0.460	0.850	0.22	0.742	0.434	0.798	0.21	0.628
	9,000	0.528	0.766	0.31	0.722	0.500	0.854	0.26	0.676	0.448	0.794	0.23	0.713
Negative Control		0.557	0.814	0.33	0.790	0.469	0.833	0.23	0.696	0.375	0.781	0.16	0.549
Positive Control		0.667	0.823	0.44	0.954	0.637	0.760	0.42	0.825	0.544	0.917	0.29	0.611

[1]nPP = Total Phosphorus – (Phytic Acid * 0.282) [28]

Table 5. Effect of CEP Product and Dose on Starter, Grower, and Finisher Period Broiler Performance

Treatment		Starter (D1-10)			Grower (D11-21)			Finisher (D22-42)		
Product	Dose (FTU/kg)	FI[1] (kg/bird)	LWG[2] (kg/bird)	FCR[3]	FI[1] (kg/bird)	LWG[2] (kg/bird)	FCR[3]	FI[1] (kg/bird)	LWG[2] (kg/bird)	FCR[3]
CEP1	3,000	0.322^{ab}	0.257^{a}	1.251	1.024^{a}	0.700^{ab}	1.463	3.625	2.058	1.761
	6,000	0.325^{a}	0.260^{a}	1.259	1.009^{ab}	0.697^{ab}	1.446	3.577	2.010	1.762
	9,000	0.323^{ab}	0.263^{a}	1.226	1.010^{ab}	0.710^{a}	1.413	3.545	1.993	1.770
CEP2	3,000	0.314^{bc}	0.253^{ab}	1.238	0.978^{b}	0.682^{b}	1.432	3.490	1.944	1.767
	6,000	0.320^{ab}	0.258^{a}	1.239	1.002^{ab}	0.705^{a}	1.421	3.483	1.926	1.809
	9,000	0.318^{abc}	0.253^{ab}	1.257	1.013^{ab}	0.708^{a}	1.430	3.543	1.977	1.776
Negative Control		0.308^{c}	0.243^{b}	1.262	0.929^{c}	0.639^{c}	1.452	3.408	1.867	1.811
Positive Control		0.324^{a}	0.259^{a}	1.251	1.026^{ab}	0.700^{ab}	1.464	3.567	1.969	1.804
P-Value		**0.0104**	**0.0290**	0.1586	<**0.0001**	<**0.0001**	0.0789	0.0747	0.0869	0.8718
SEM[4]		0.0035	0.0039	0.0100	0.0134	0.0069	0.0137	0.0483	0.0420	0.0326
Product Means										
CEP1		0.324^{a}	0.2591	1.245	1.014	0.702	1.441	3.582	2.020^{a}	1.764
CEP2		0.317^{b}	0.2547	1.245	0.998	0.698	1.428	3.506	1.949^{b}	1.784
Product SEM[4]		0.0020	0.0020	0.0105	0.0084	0.0040	0.0081	0.0287	0.0231	0.0198
Dose Means										
3,000 FTU/kg		0.318	0.255	1.244	1.000	0.691^{b}	1.448	3.558	2.001	1.764
6,000 FTU/kg		0.323	0.258	1.249	1.005	0.701^{ab}	1.433	3.530	1.968	1.785
9,000 FTU/kg		0.321	0.257	1.241	1.011	0.709^{a}	1.421	3.544	1.985	1.773
Dose SEM[4]		0.0025	0.0030	0.0128	0.0103	0.0050	0.0010	0.0352	0.0282	0.0242
Probability										
Product		**0.0298**	0.1297	0.9346	0.1803	0.4916	0.2689	0.0636	**0.0328**	0.4745
Dose		0.4109	0.6836	0.7285	0.7610	**0.0330**	0.1833	0.8576	0.7160	0.8196
Product x Dose		0.8315	0.4267	**0.0275**	0.2139	0.1834	0.2035	0.4004	0.4520	0.7878

[a-c] Means within the same column with no common superscript differ significantly (P<0.05)

[1] FI = Feed intake per bird

[2] LWG = Live Weight Gain per bird

[3] FCR = =Mortality corrected feed conversion ratio

[4] SEM = Pooled standard error of the mean

Figure 1. Interaction plot between product and dose using FCR as response for d1-10.

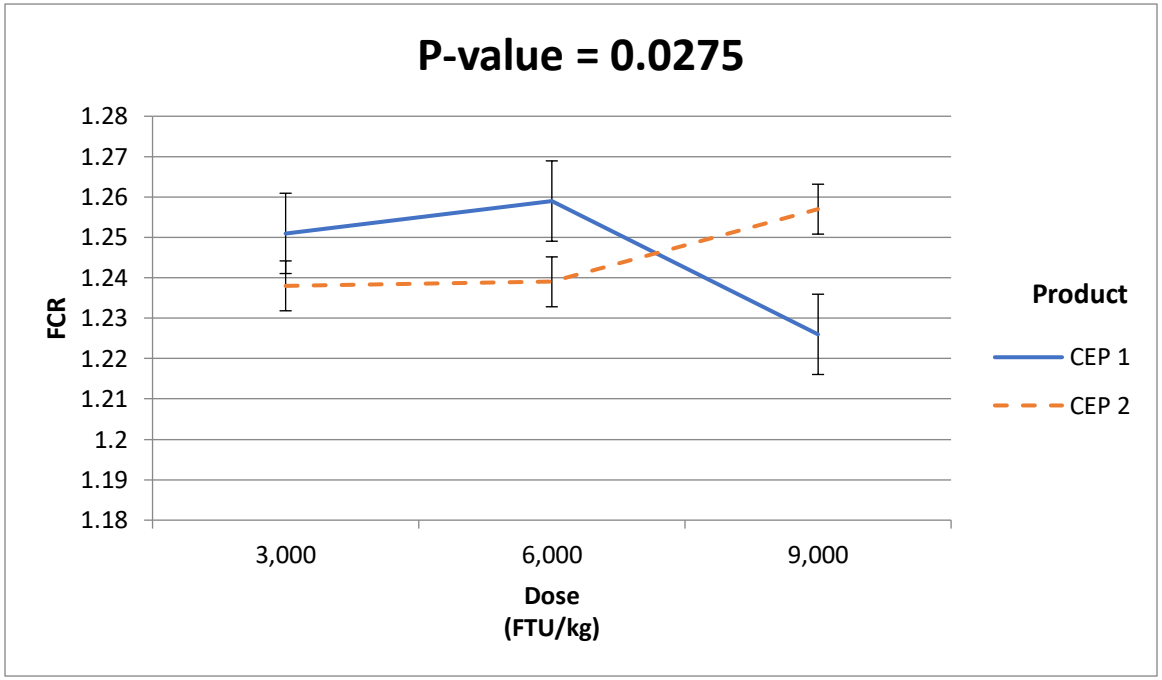

Table 6. Effects of CEP Product and Dose on Overall Period (D1-42) Broiler Performance

Product	Dose (FTU/kg)	Avg. Body Weight (kg)	FI[1] (kg/bird)	LWG[2] (kg/bird)	FCR[3]	Corrected Mortality[4] (%)
CEP1	3,000	3.047[a]	5.376[a]	3.006[a]	1.625	4.386
	6,000	3.001[a]	5.322[a]	2.960[a]	1.621	5.263
	9,000	3.005[a]	5.286[a]	2.965[a]	1.611	4.825
CEP2	3,000	2.924[a]	5.211[ab]	2.883[a]	1.617	6.140
	6,000	2.930[a]	5.212[ab]	2.889[a]	1.631	3.509
	9,000	2.979[a]	5.290[a]	2.938[a]	1.623	4.825
Negative Control		2.792[b]	5.034[b]	2.751[b]	1.653	5.263
Positive Control		2.980[a]	5.321[a]	2.938[a]	1.648	4.825
P-Value		**0.0063**	**0.0257**	**0.0064**	0.6968	0.9059
SEM[5]		0.0443	0.0676	0.0444	0.0181	1.2137
Product Means						
CEP1		3.018[a]	5.328	2.977[a]	1.619	4.825
CEP2		2.944[b]	5.237	2.903[b]	1.624	4.825
Product SEM[5]		0.0241	0.0421	0.0242	0.0110	0.6656
Dose Means						
3,000 FTU/kg		2.985	5.294	2.945	1.621	5.263
6,000 FTU/kg		2.966	5.267	2.925	1.626	4.386
9,000 FTU/kg		2.992	5.288	2.951	1.617	4.825
Dose SEM[5]		0.0296	0.0516	0.0296	0.0134	0.8152
Probability						
Product		**0.0351**	0.1338	**0.0352**	0.7563	1.000
Dose		0.8050	0.9300	0.8032	0.8981	0.7498
Product x Dose		0.5183	0.4994	0.5215	0.8324	0.3217

[1] D1-42 FI = D1-21 FI/bird + D22-42 FI/bird. This corrected the measurement for the removal of 5 birds per pen for tibia excision on D21.
[2] D-1-42 LWG = D1-21 LWG/bird + D22-42 LWG/bird. This corrected the measurement for the removal of 5 birds per pen for tibia excision on D21.
[3] D1-42 FCR was corrected for mortality, which included the weight of the three birds per pen removed for tibia analysis on D21.
[4] Corrected Mortality = ((100- ((D42 Bird #/D1 bird #) * 100)) – ((5/24)*100))). This corrected the measurement for the removal of 5 birds per pen for tibia excision on D21
[5] Pooled standard error of the mean.

Table 7. Effect of CEP Product and Concentration on D21 and D42 Tibia Ash

Treatment		D21		D42	
Product	Dose (FTU/kg)	Tibia Ash[1] (%)	mg Tibia Ash/bird[2] (mg/bird)	Tibia Ash[1] (%)	mg Tibia Ash/bird[2] (mg/bird)
CEP1	3,000	48.40^{c}	986.02^{bc}	48.25^{a}	3398.06^{abc}
	6,000	48.81^{abc}	1053.57^{a}	48.27^{a}	3509.86^{a}
	9,000	49.10^{a}	1036.67^{ab}	48.33^{a}	3461.70^{ab}
CEP2	3,000	48.54^{bc}	948.98^{c}	48.32^{a}	3305.97^{bc}
	6,000	48.87^{ab}	1039.73^{a}	48.14^{a}	3248.36^{c}
	9,000	48.86^{ab}	1040.50^{a}	48.50^{a}	3452.86^{ab}
Negative Control		45.14^{d}	739.47^{d}	46.95^{b}	2747.25^{d}
Positive Control		48.90^{ab}	1001.92^{ab}	48.60^{a}	3351.22^{abc}
P-Value		**<0.0001**	**<0.0001**	**0.0009**	**<0.0001**
SEM[3]		0.1501	18.6280	0.2564	66.6933
Product Means					
CEP1		48.77	1025.42	48.28	3456.54^{a}
CEP2		48.75	1009.74	48.32	3335.73^{b}
Product SEM[3]		0.0840	11.1910	0.1391	37.8502
Dose Means					
3,000 FTU/kg		48.47^{b}	967.50^{b}	48.28	3352.01
6,000 FTU/kg		48.84^{a}	1046.65^{a}	48.20	3379.11
9,000 FTU/kg		48.98^{a}	1038.58^{a}	48.41	3457.28
Dose SEM[3]		0.1023	13.7062	0.1702	46.3562
Probability					
Product		0.8866	0.3262	0.8576	**0.0280**
Dose		**0.0024**	**0.0002**	0.6846	0.2576
Product x Dose		0.3904	0.5750	0.8149	0.1550

[a-d] Means within the same column with no common superscript differ significantly (P<0.05)

[1]Tibia ash percentage was determined on dry, fat-extracted tibiae excised the from the left leg of 21d (5) or 42d (3) broilers.

[2]mg tibia ash per bird was determined by dividing the weight (mg) of the tibia ash by the number birds utilized for tibia ash determination.

[3] SEM = Pooled standard error of the mean

CURRICULUM VITAE

Niles R Ridgeway

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Education

Master of Science, Nutrition and Food Science **January 2018- December 2019**
West Virginia University, Morgantown, WV
Current GPA: 3.67

Bachelor of Science in Agriculture, Animal Science **August 2015-May 2017**
West Virginia University, Morgantown, WV
Undergraduate GPA: 3.52
Graduated with CUM LAUDE honors

Associate of Arts, General Studies **August 2013-May 2015**
Potomac State College, Keyser, WV
Associate GPA: 3.68

Academic Experience

Graduate Research Assistant **January 2018-May 2019**

- **Primary graduate student assigned to**
  - **DSM contract study evaluating direct-fed microbials in a naturally challenged environment as part of thesis project(May-July 2018)**
  - **Agrivida contract study evaluating phytase activity concentration in grain effects mixer homogeneity, thermal stability post-pellet, and efficacy in broiler performance and tibia ash (Nov-Jan 2018**
- Graduate student assigned to assist with
  - Yara International contract study evaluating different Inorganic Feed Phosphates and their Ca/P digestibility (Jan 2018)
  - Agrivida Inc. contract Study utilizing corn expressed carbohydrase(March 2018)
  - *E.faecium* mitigation project utilizing new equipment from WVU Pilot Feed Mill Renovation (March 2018)
  - Agrivida Inc. contracted study utilizing corn expressed phytase (April 2018)
  - Contract study with DSM Nutritional Products, Inc. evaluating thermal stability of multiple enzymes and effect on feed manufacture metrics (April 2018)
  - Summer Undergraduate Research Experience (SURE) project determining synthetic methionine inclusion of organic broiler diets to benefit production or health(May-July 2018)
  - Contract study with Huvepharma, Inc, evaluating a new pellet binder (May-June 2018)
  - Feed manufacture for digestibility study utilizing turkey poults with Penn State University (July 2018)

        - Thermal stability contract study with JBS (United) enzymes (August 2018)
        - Contract study with Huvepharma, Inc, evaluating thermal stability of enzyme cocktails (August 2018)
    - Appointed Financial Manager for all accounts of lab group(April 2018-Dec 2018)
        - File receipts, reconcile credit cards, monitor grant funds

Professional Development

- Poultry Science Association Annual Meeting, San Antonio, TX (2018)
    - Accepted 1-year position as University Ambassador for National Student Organization
- Arkansas Nutrition Conference, Rogers, AR (2018)

Teaching Experience

- Guest Lecturer
    - A&VS 150 (Intro to Animal Science)(Feb 2018)
        - Conducted presentation on dairy production at a nearby farm of previous employment
    - A&VS 251 (Principles of Animal Science)(Aug 2018)
        - Brief lecture/tour of pilot feed mill explaining feed manufacture and current research focus of program
- Assist with Poultry Evaluation course ANPR 339 (Feb 2018-April 2018)
    - Prepare students for collegiate contest in Baton Rouge, LA
- **Primary Teaching Assistant for ANPR 369** (Poultry Production Lab)(Aug 2018-Dec 2018)
    - Advise students on poultry production, specifically broilers from egg to table
        - Vaccinations, collection of blood samples, general rearing strategies, feed manufacture for multi-phase growth
- Social Welcome Back BBQ for Davis College
    - Prepare/Deliver Food
- Undergraduate Teaching Assistant
    - A&VS 410 Dairy Heifer Management
    - A&VS 150 Intro to Animal Science
    - ANPR 343 Beef Production Lab

Extension Experience

- Wyoming County Project (Feb, Mar, May 2018)
    - Series of visits with presentations on rearing backyard poultry for egg production targeting housing, nutrition, and marketing
- Backyard Poultry Dinner Meeting (Hico, WV; Feb. 5 2018)
- Assisted with 4H Poultry Judging Competition during WV Poultry Festival in Moorefield, WV, 7/18/18)
- Poultry Judge for 4H fair projects
    - Garrett County Fair (McHenry, MD) (July 2018)
    - Monongalia County Fair (Morgantown, WV) (August 2018)
- State FFA CDE Poultry Evaluation (Morgantown, WV) (2018)

        - Set-up and host state contest utilizing university facilities and poultry(Sept 18)
    - Additional practice for winning FFA team (Oct 18)
        - Set-up additional rings of competition and explain placing of products, assisting/preparing the team for the National CDE event

Academic Awards/Honors/Scholarships

- Member of American Legion Mountaineer Boy's State (2012)
- Lynch Memorial Scholarship x2 (2013-2015)
- PROMISE Scholarship(2013-2017)
- Potomac State College Provost's List (later changed to President's) (Fall 2013)
- Potomac State College Dean's List (Spring 2014)
- Potomac State College President's List (Fall 2014, Spring 2015)
- Resident Transfer Scholarship (2015)
- 3 Little Pigs Scholarship (2015)
- Member of Collegiate Poultry Judging Team (Spring 2017)
    - Obtained 5th place as a team at the national contest in Baton Rouge, LA
- WVU Dean's List (Spring 2017)

Animal/Scientific specific courses taken

- Undergraduate Courses
    - A&VS 251 Principles of Animal Science
    - A&VS 411 Dairy Heifer Management
    - AGBI 410 Intro to Biochemistry
    - ANNU 260 Intro to Animal Nutrition
    - ANNU 362 Applied Nutrition 2 (Non-Ruminant)
    - ANPR 350 Milk Production
    - ANPR 338 Poultry Judging
    - ANPR 341+343 Beef Production w/lab
    - ANPR 367+369 Poultry Production w/lab
- Graduate Courses
    - STAT 511 Statistical Methods I
    - AGBI 512 Nutritional Biochemistry
    - A&VS 797 Research
    - STAT 512 Statistical Methods II
    - STAT 513 Design of Experiments
    - FDST 545 Food Microbiology

Job Experience

Broiler Service Technician **May 2019-PRESENT**

Pilgrim's, Moorefield, WV

- Poultry integrator located globally and process 2 million head per week at current complex
- Responsible for 1 million square foot of contract growing barns
- Act as a liaison between company and contract growers

- Monitor bird health, management strategies, and feed inventories to ensure proper animal welfare is maintained whilst also maintaining adequate performance for profitability
- Adapt to several work environments
- Track, record, and analyze bird mortality rates and identify issues within the GI tract to identify cause of mortality

Production Manager **May 2017-December 2017**

Orr Ag, LLC, Belle Vernon, PA

- 200-head dairy farm in southwestern Pennsylvania that also raises 800 acres of crops, both for forage and cash crop purposes
- Often work unsupervised on various projects including crop work, building maintenance, livestock handling, and transportation(grains, feed, or livestock)
- Adapt to several work environments
- Maintain vehicles for on-road transportation with routine service intervals
- Use and analyze animal records daily through DairyComp305
- Primary Artificial Insemination technician while employed
- Work with Veterinarian, Nutritionist, and Semex Rep to construct a plan for optimal production, while maintaining healthy and sound herd

Agriculture Laborer **May 2015- May 2017**

Teets Cattle Company, Lost River, WV

- Commercial cow-calf operation with 850 brood cows
- Worked independently often, however some harvesting days involved a team
- Upkeep on facilities and grounds
- Provide animals with fresh bedding, feed, and water
- Transport animals to pasture or to market
- Assist with Estrus Detection and Artificial Insemination, as well as embryo transfer synchronization programs
- Harvest crops for high RFV(Relative Feed Value)
- Manage both the spring-calving herd and fall-calving herd
- Select and raise club calves strategically for local 4h and FFA members to purchase
- Serviced vehicles/tractors and made repairs when needed(metal fabrication/welding, filter changes, part replacement)

Agriculture Laborer **May 2014-August 2014**

Branson Farms, LLC, Lost River, WV

- 300-head beef cow-calf operation
- 15 commercial poultry houses with contracts through the Virginia Poultry Growers' Cooperative to raise male turkeys(toms) through 19 weeks
- Monitored bird health, feed/water, housing conditions, and practiced biosecurity from flock-to flock
- Often renovated/retrofitted different houses to modernize outdated equipment to ensure profitability of each flock

Skills

- Microsoft Office Proficiency
- Mechanical inclination
- Adaptability to work in all environments
- Ability to work independently or as part of a team/group
- Husbandry experiences with all forms of livestock
- Problem-solver
- Critical-thinking
- Feed manufacture
- Poultry market judging
- Research data collection
- Commercial Agriculture knowledge