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FEED CONDITIONING EFFECTS ON ENZYMES, AMINO ACIDS, AND SUBSEQUENT BROILER PERFORMANCE

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ABSTRACT

Feed Conditioning Effects on Enzymes, Amino Acids, and Subsequent Broiler Performance

Elizabeth Lynch

There are many things that can be done to improve broiler performance including, but not limited to, pelleting, proper Lys inclusion, and exogenous enzyme supplementation. Pelleting has the ability to improve feed form, reduce wastage, improve bird performance metrics, and decrease pathogens within the feed. Pathogen reduction can be further reduced by hygienic pelleting practices which include an increase in temperature or retention time within a conditioner or hygieniser. However, hygienic pelleting can degrade valuable AA and enzymes (more specifically for the current thesis research Lys and phytase) and make them unavailable to the animal. Supplementing Lys to account for the loss can increase the price of feed production but can be balanced by the improvement in broiler LWG. Performing research on novel phytases to improve enzyme activity after the addition of heat and within the digestive tract of the bird critical in order to improve bird performance metrics.

Evaluating high conditioning temperature and exposure time within the pelleting process (hygienic pelleting) in diets that vary in digestible lysine and corresponding amino acid ratio on Ross 708 starter broiler performance, amino acid digestibility, and requirement is the purpose of the initial study. A 5 x 2 factorial arrangement of treatments with 5 levels of digestible lysine (-20%, -10%, 2019 Ross Broiler Starter Nutrition recommendation, +10%, and +20%) and 2 degrees of pelleting (Standard and Hygienic) was utilized in a randomized complete block design. Each treatment was fed to 12 replicate pens of 10 male broiler Hubbard x Ross 708 chicks for a 21-day period. On the morning following day 7, 14 and 21, birds and feed were weighed by pen for performance calculations and ileal contents were collected on d22 for AA digestibility calculations. Standard Pelleting and Hygienic Pelleting demonstrated a 1.19 and 1.37% Lys requirement, respectively, using the broken-line regression model. Performance data and the broken-line regression model suggest an approximate 10% increase in Lys and corresponding amino acid ratio relative to 2019 Ross Broiler Starter Nutrition Specifications for Hygienic Pelleting to provide optimal live weight gain. Hygienic Pelleting decreased amino acid digestibility and increased subsequent FCR when diets were formulated to decreased amino acid density.

The assessment of novel exogenous phytase enzymes that can be added to the mixer prior to pelleting is important for the continual improvement of global broiler production. Two experiments were conducted to determine *in vitro* activity post pelleting and *in vivo* efficacy post feeding of a novel phytase. The objective of the first study was to determine activity and retention of a novel phytase enzyme (CJ Phytase) and a commercially available phytase (Quantum Blue 5G®) at different 30 second conditioning temperatures (75, 80, 85, and 90˚C) post-pelleting. The objective of the second study was to determine the effects of these phytase sources conditioned for 30 seconds at the 75˚C conditioning temperature on broiler performance, bone mineralization, and mineral digestibility. Experiment one consisted of a 2 (phytase source) x 4 (conditioning temperature) factorial arrangement. A conditioning temperature main effect ($P=0.0003$) demonstrated that activity of both phytase products decreased at 85˚C and decreased again at 90˚C. In experiment two, 2,304 Hubbard x Ross 708 birds were obtained and fed one of

eight diets for a 42-day period. Diets included Positive Control, Negative Control deficient in calcium and Available P by 0.2%, and graded levels of NC+ CJ Phytase (250, 500, 1,000, 1,500, and 3,000 FTU/kg). In order to provide a commercially available comparison, an NC + Quantum Blue 5G 500 FTU/kg was also fed. All phytase additions increased AID P, d42 tibia ash percentage, and d0-42 LWG relative to the NC diet ($P<0.05$). CJ Phytase above 500 FTU/kg and Quantum Blue 500 FTU/kg increased AID Ca relative to the NC diet ($P<0.05$). The novel CJ phytase demonstrated efficacy in post pellet retention, mineral digestibility, tibia ash percentage, and d0-42 broiler performance.

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ABBREVIATION KEY

Chapter 1

1. Feed conversion ratio- FCR
2. Live weight gain- LWG
3. Amino acids- AA
4. Lysine- Lys
5. Cysteine- Cys
6. National Research Council- NRC
7. Phosphorous- P
8. Soybean Meal- SBM
9. Non-Phytate Phosphorus- nPP
10. Dicalcium phosphate-DCP
11. Monocalcium phosphate-MCP
12. Calcium- Ca
13. Copper- Cu
14. Zinc- Zn
15. Manganese- Mn
16. Iron- Fe
17. Phytase Units- FTU
18. Positive Control- PC
19. Negative Control- NC

Chapter 2

1. Amino Acid- AA
2. Lysine- Lys
3. Digestible- dig
4. Pellet Durability Index- PDI
5. Dry Matter Percent- DM%
6. New Holmen Pellet Tester- NHPT
7. Live Weight Gain- LWG
8. Feed Intake- FI
9. Feed Conversion Ratio- FCR
10. Individual Bird Weight- IBW
11. Apparent Ileal Digestibility- AID
12. Digestible Amino Acid Concentration- DAAC
13. Analysis of Variance- ANOVA
14. Least Significant Difference- LSD
15. Broken-Line Regression Model- BLRM
16. National Research Council- NRC
17. Advanced Glycation End-Products- AGEs
18. High-Performance Liquid Chromatography- HPLC

Chapter 3

1. Phosphorous- P
2. Soybean Meal- SBM
3. California Pellet Mill- CPM
4. Positive Control- PC
5. Negative Control- NC
6. Calcium- Ca
7. Live Weight Gain- LWG
8. Feed Intake- FI
9. Feed Conversion Ratio- FCR
10. Individual Bird Weight- IBW
11. Apparent Ileal Digestibility- AID
12. Analysis of Variance- ANOVA
13. Least Significant Difference- LSD
14. Pellet Durability Index- PDI

CHAPTER ONE

LITERATURE REVIEW

1.Feed Manufacture

1.1 Pelleting

Pelleting is the process of taking ground, mixed feed ingredients and extruding through a pellet die with the help of steam, pressure, and heat. This process has been shown to improve many facets of the livestock industry including, but not limited to, handling, transportation, storage of feed, feed hygienics, and increased animal health. Pelleting will decrease ingredient segregation, energy expenditure by the animal during prehension, and feed spillage [8, 19]. The benefits of pelleting come together to produce a feed that improves performance metrics of animals such as a decreased feed conversion ratio (**FCR**) and increased live weight gain (**LWG**) [19, 69].

The pelleting process begins with the grinding of large particle size ingredients such as corn, wheat, barley, cereal grains etc. into a finer particle. In order to do so, a hammer or roller mill may be utilized [1]. A hammer mill uses impaction to grind the feed, is versatile as it can process many different materials, and is inexpensive to purchase/maintain. However, it has a large standard deviation for particle size. A roller mill utilizes compaction to grind, provided a better electrical efficiency, and has a smaller standard of deviation for particle size. Yet, a roller mill is expensive to obtain.

From this point, other micro ingredients such as crystalline amino acids (**AA**), vitamins, minerals, fat (in the form of soybean oil for the West Virginia Pilot Feed Mill), and exogenous enzymes can be blended in with the macro ingredients. This mixture is referred to as mash. Mash

can be fed to animals without further processing. However, studies have shown that broilers perform at a higher level when fed pellets due to the aforementioned benefits when compared to broilers feed an all-mash feed [64]. Birds, similar to any animal, can be picky. Birds tend to become satiated on large particle size or more palatable ingredients and do not receive the nutritional effects of the diet as a whole. Thus, feeding pellets will limit selective feeding. Feed wastage is also extremely high with all-mash diets. Therefore, in order to optimize bird performance and reduce waste, it is suggested that pellets be fed [64].

After the ingredients have been blended, the mash feed should be conditioned or moistened in order to have the capacity to form a pellet. Moritz, et al. found that the addition of moisture at the mixer tended to enhance pellet durability and decrease pellet mill energy consumption due to the added lubrication at the die [56]. Moisture can also be added through the conditioner. A conditioner increases the temperature of the mixed mash and adds moisture via steam. Average conditioning temperature varies from 70-90°C. For example, The West Virginia University Pilot Feed Mill has a standard conditioning temperature of 77°C for 30 seconds. However, changes in location will affect the "standard'. This variability is due to ambient temperature and humidity as well lipid and starch content of the feed [18, 20]. However, commercial feed manufacture practices and past research does not agree on a set optimal conditioning temperature [26].

The mixed mash can also be subjected to a hygieniser. The hygieniser is a cylindrical vessel that maintains conditioning temperature through use of a jacketed heating system and is typically located between the conditioner and press feeder in a feed mill [12]. While pelleting has a slight effect on feed hygienics, a short-term conditioner is not sufficient for decontamination of pathogens [61]. However, the reduction of pathogens can be achieved with the use of a

hygieniser. Unlike the conditioner which adds steam to the mixed mash, the hygieniser maintains feed temperature for an extended time to reduce the prevalence of harmful microorganisms like *Salmonella*. The retention times range from 4 seconds to 6 minutes. Numerous serotypes of *Salmonella spp*. have been isolated from feed mills, with *Salmonella typhimurium* and *Salmonella enteritidis* sampled frequently [27]. The *Salmonella spp*. can then infect human and animals, and, in this case, poultry. Therefore, it is imperative further action be taken to reduce the prevalence of the *Salmonella spp.* within feed and the application of a hygieniser has the capability to do so.

After conditioning, the mash is pressed between a roller and die, extruded through a pellet die, and cut uniformly. Because of sheer forces, heat caused by friction can increase as the pellets move through the die. Tumuluru found a 10% decrease in feedstock moisture content after pelleting due to frictional heat developed in the pellet die, which can result in moisture flash off as the pellet is extruded [70]. After pelleting, pellets must be cooled with a fan or cooling deck in order to reduce pellet moisture which ultimately decreases the chance of fungal growth. The addition of moisture to diets may produce negative effects such as nutrient dilution and mold spore proliferation [41].

1.2 Feed Hygienics

High value is placed on broiler breeders due to the large financial, time, and resource investment. Precautionary steps can be taken during the feed manufacture process to support broiler health and safety, ultimately protecting the production investment. Feed costs account for 60-65% of the total expense for broiler production [26]. One precautionary step includes increased conditioning temperature and time to reduce pathogenic bacteria loads that may be

associated with feed or feed ingredients. Feed is subjected to increased temperature and retention time in the conditioner and increased time retained in a hygieniser. Studies have shown many benefits to pelleting feed with increased temperatures and retention time. Increasing conditioning temperature has been shown to improve pellet quality and durability [15, 43]. The addition of heat has the ability to denature and dissociate the structure of proteins. Therefore, making pellets more digestible [52]. Adding heat to non-starch polysaccharides can also break down the aleurone layer of cell walls which encapsulate a high amount of nutritive components. The breakdown of the aleurone layer ultimately makes the nutrients more available [10]. Furthermore, increased retention time in a conditioner or in a hygieniser, can lead to better efficacy of hydrothermal treatment in terms of decontamination [13, 17]. Boltz, et al. found a 3- and 4-log reduction of *Enterococcus faecium* (a *Salmonella* surrogate) when evaluating the differences between two pelleting techniques: standard pelleting of 70°C for 15s without the hygieniser and a more thermally aggressive pelleting of 80°C for 30s with a 45s retention time in the hygieniser [12]. Another study done by Boney, et al. concluded that using short-term steam conditioning of 10 seconds demonstrated a 3-log reduction in the *Salmonella* surrogate *E. faecium*, while long-term steam conditioning of 60 seconds resulted in a 4-log reduction [13].

Conversely, hygienic, or hydrothermal, pelleting can cause issues to arise because of the increase in heat exposure. Hygienic pelleting has been known to cause protein denaturation in excess, which can delay the digestion of proteins through reduction in protein solubility as well as provide the necessary environment for Maillard reactions [5, 22, 24]. Loar, et al. reported that conditioning corn and SBM-based diets above 74°C increased broiler FCR and decreased AA digestibility [50]. The inclusion of enzymes, such as phytases, can also be affected by an increase in temperature or retention time. Homen, et al. [39] concluded that as conditioning temperature

increased, *in vitro* phytase activity decreased after steam-conditioning diets containing corn and SBM at temperatures 82°C, 88°C, and 93°C ($P<0.05$).

Due to these atypical thermal processing conditions, it is common for diets to be over-formulated in amino acids or supplemented enzymes to account for any denaturation that may occur. Losses occur as a result of the increased feed processing temperatures and are prominent with heat-labile AA such as Lys (**Lys**) and Cystine (**Cys**) [59]. Including densities over requirement for expensive ingredients, such as amino acids, is economically unfavorable. Therefore, the over-formulation should be based on comprehensive data.

2. Dietary Constituents and Feed Additives

2.1 Lysine

Lysine (2,6-diaminohexanoic acid) is comprised of a positively charged R group [62]. It contains a reactive ϵ-amino group, is the most chemically reactive amino acid, and is susceptible to chemical modification during processing and prolonged storage [57]. Increasing Lys content over National Research Council (**NRC**) levels was reported to improve FCR, breast meat yield, and BWG with low abdominal fat pad weight [47]. Lysine is responsible for an increase in meat production as well as broiler efficiency [42]. Holsheimer and Ruesink [38] showed that breast meat yield was increased in male broilers fed diets containing increasing Lys levels from 1-14 days of age. Another study done by Kidd, et al. showed that feeding broilers above NRC recommendations improved broiler performance and breast meat traits [44]. Additionally, Lys is involved in many critical metabolic and physiological processes such as the production of somatic proteins, synthesis of ketone bodies, and metabolism of glycolipids [9, 37, 49]. Lys is the second limiting amino acid in practical corn-SBM poultry diets and is vital to many bodily

processes/the improvement of bird performance therefore, Lys is crucial for poultry producers and feed manufacturers to be concerned with the amount of digestible Lys in the diets.

2.2 Maillard Reaction

The Maillard reaction is important when evaluating the reactiveness of the AA Lys. The Maillard reaction is related to aroma, taste, and color change after heat addition, more commonly talked about regarding baking products and meat [63]. As previously mentioned, heat and moisture are applied to feed during the pelleting process through the application of steam. The pelleting process provides the necessary conditions to induce a Maillard reaction that would render Lys, the seconding limiting AA in poultry diets, unavailable. The reaction itself is a highly complicated process. This reaction is initiated by heat and begins with the interaction between a carbonyl group of a reducing sugar and an amino compound, followed by rearrangement and production of an amadori product [55]. The end products of Maillard reactions are enzymatically undegradable, leading to a reduction in protein, AA, and carbohydrate availability [5, 34]. Fontaine and coauthors [35] noted that when heated in an autoclave at 135℃, SBM commonly experienced 10-20% Lys damage, and when overheated could easily lose up to 67% reactive Lys. Another study by González-Vega utilized an autoclave at 125°C to heat SBM. As exposure time increased to 30 minutes, ileal digestibility of AA in growing pigs decreased linearly ($P< 0.01$) due to the Maillard reaction [36].

2.3 Phytate

Phosphorus (**P**) is the second most abundant mineral in the body of the bird and about 80% of P is stored as hydroxyapatite in the bone [2, 72]. It is responsible for the growth and strength of the skeletal system [14]. Additionally, it is a necessary component of nucleic acids in addition

to phospholipids in cell membranes [30]. Studies show that 2/3 of the P found in poultry diets is bound to phytate P as poultry diets are primarily made up of the macro ingredients corn and soybean meal (**SBM**) which are high in phytate P content [58]. The other 1/3 consists of non-phytate phosphorus (**nPP**) which is available for the animal to utilize. Phytate P (myo-inositol hexakisphophate, IP6) also serves as the primary storage form of P in cereal grains and oilseeds [33, 53]. Phytate P is the most abundant form of P in plant-based animal feeds. Although it is abundant, it is considered an anti-nutritive factor. Monogastric animals, such as poultry, are unable to utilize and digest phytate P due to the lack of a dietary enzyme, phytase [74]. A reduction in available P can impede broiler performance as P is responsible for skeletal development [14]. Because phytate P is not absorbed, it is excreted from the bird thus presenting a potential environmental challenge. To further exacerbate the problem, in order for broilers to receive enough P, inorganic P in the form of dicalcium phosphate (**DCP**) and monocalcium phosphate (**MCP**) is often supplemented in feed [29]. Excess P from the excreta and litter will collect in water run-off which leads to eutrophication of surface waters in nearby waterways as well as increased fish-kills [30]. Furthermore, phytate has the ability to chelate with vital minerals such as such as **Ca, Cu, Zn, Mn,** and **Fe** making them unavailable to the bird and therefore indigestible [11].

2.4 Phytase

These challenges presented by phytate P can be overcome with the inclusion of the highly valuable exogenous enzyme, phytase, in poultry diets. [6] Phytase is used in commercial broiler diets to improve P availability, diet cost, and environmental impact [7, 65]. The enzyme (myo-inositol hexaphosphate phosphohydrolases) catalyzes the hydrolytic cleavage of the phosphoric acid esters of inositol, which release P [48]. In lay terms, phytase is responsible for hydrolyzing

phytate P and liberating phosphorous [4]. Selle and Ravindran report that dietary inclusions of phytase feed enzymes generates bioavailable P and reduces the P load within the environment [65]. Enzymatic activity of phytase is measured in phytase units (**FTU**) and is defined as the amount of enzyme required to release 1 μmol of inorganic phosphate per minute from a 5.1 mM sodium phytate at pH 5.5 and 37 ˚C [32].

The addition of phytase not only improves the absorption of P, but also other minerals that may have chelated with phytate P [60]. Because phytase has the ability to increase P and mineral digestibility, it is also credited with improving tibia ash percentage, bone breaking strength, and broiler performance [25]. Campasino and cohorts [21] performed a study using treatments consisting of a positive control (**PC**), a Ca and P deficient negative control (**NC**), NC + 400, NC + 800, NC + 1,200, and NC + 1,600 FTU of phytase. They found that the supplementation of phytase decreased FCR linearly. Broilers fed NC + 1,600 FTU of phytase had lower FCR and higher breast meat weight than those fed the PC diet. A study done comparing Optiphos® and Optiphos Plus® (a more heat tolerant phytase from its predecessor) showed that broiler performance metrics continued to improve up to approximately 1,500 FTU/kg [4]. Another concept that must be mentioned is super-dosing phytase. Inclusions greater than 1,500 FTU/kg are considered super-dosing [14]. Shirley and Edwards supplemented corn-soybean meal diets with 93 FTU/kg and 12,000FTU/kg. A quadratic increase was seen in phytate-P disappearance with increasing phytase dose (0, 93, 187, 375, 750, 1,500, 3,000, 6,000, or 12,000 FTU/kg) from around 42% to almost 95%, respectively. Birds given the high dose of phytase were also able to utilize an additional 200 ME/kg diet which suggests that the inclusion of phytase at higher levels may influence more than mineral utilization [66]. Super-dosing can also help with the phytase losses due to thermal processing.

Phytase inclusion in the diets can be completed by one of two ways: post-pellet application and mixer-added inclusion. The post-pellet application method requires costly equipment and poses concerns about uniform application [7]. To bypass the added expenses and ensure uniformity, mixer-added phytase inclusion is common. However, this type of phytase has the potential of becoming denatured during the pelleting process as phytase must be exposed to increased temperatures and moisture [51]. Companies that develop mixer-added phytase enzymes are interested in developing a heat-stable product that retains activity post-pelleting. Homen et al [39] concluded that as conditioning temperature increased, *in vitro* phytase activity decreased after steam-conditioning diets at temperatures 82°C, 88°C, and 93°C. In addition to retention, functionality of phytase within the digestive tract must be maintained as phytases are sensitive to pH and can be denatured by proteases [54]. Therefore, *in vivo* testing of the enzyme must be performed [51].

2.5 Phytase Products

Typically, phytase products are produced from microorganisms such as bacteria, yeast, and fungi [68, 73]. Phytase is a protein and is susceptible to hydrolysis by endogenous proteases found within the digestive tract of poultry [18, 28]. Depending on the source of the phytase product, the ability of the phytase to withstand hydrolysis from proteases is variable. Therefore, it would be prudent to determine the functionality of any novel phytase within the digestive tract through extensive *in vivo* research before recommending the product to consumers. It has been observed that the new-generation bacterial phytases from *Escherichia coli* have a very specific affinity for IP6 and IP5 bond. They also possess higher resistance to proteolytic digestion than fungal phytases from the common *Aspergillus niger*, which may partly explain their higher

efficacy reported in trial studies [3, 28]. To prove this point, a study examined the reaction of three different phytases incubated in a buffer containing protease. *Escherichia coli* was shown to have a higher resistance than both fungal phytases *Peniophora lycii* and *Aspergillus niger* [46].

As previously mentioned, phytase is also sensitive to pH levels. Thus, ensuring phytase is not degraded within the gastrointestinal tract of the monogastric animal is a necessity when testing novel phytases. Various studies have altered phytases to resist degradation at low pH levels. For example, Shivange, et al. conducted a study in which protein consensus-based surface engineering was used to change the surface of a phytase enzyme from *Yersinia mollaretii*. The surface-engineered phytase exhibited 3.8-fold higher pH stability at pH 2.8 for 3 hours compared to the non-engineered phytase [67]. Another study by Kim, et al. shifted the optimal pH of a phytase from *Aspergillus niger* to match the stomach conditions (3.5 pH) by substituting amino acids in the substrate-binding site with different charges and polarities. They were ultimately able to improve the function of the phytase under stomach conditions with protein engineering [45].

Innovations in biotechnology have led to the study of various cereal grains and plant sources, along with their capabilities to express enzymatic activity [16]. Hong et. al. expressed two bacterial phytases in germinated transgenic rice seeds which yielded high activity over broad pH ranges. Therefore, it was determined that the two phytases could potentially be an ideal feed additive for improving the phytate-phosphorus digestibility in monogastric animals [40]. Another study done by Chen and cohorts over-expressed the *Aspergillus niger phyA2* gene in maize to increase phytase activity. The study found that phytase activity in transgenic maize seeds reached approximately 2,200 units per kg seed which is a 50-fold increase compared to non-transgenic maize seeds [23].

3. Conclusions

In conclusion, there are many methods to improve broiler performance including, but not limited to, pelleting, proper Lys inclusion, and exogenous enzyme supplementation. Pelleting has the ability to improve feed form, reduce wastage, improve bird performance metrics, and decrease pathogens within the feed. Pathogen reduction can be further reduced by hygienic pelleting practices which include an increase in conditioning temperatures and retention times or the use of a hygieniser. However, hygienic pelleting can degrade valuable AA and enzymes (more specifically for the current thesis research Lys and phytase) and make these unavailable to the animal. Supplementing Lys and super-dosing phytase to account for the loss can increase the price of feed production but can be balanced by the improvement in broiler LWG, FCR, breast tissue accretion, and bone mineralization.

4. Future Research

The study of novel phytases is recommended as there will always be a need to reduce the excretion of P by the bird thereby, reducing the environmental impact from poultry production. This is extremely important for the state of West Virginia as run-off from the state contributes to the Chesapeake Bay watershed. With broiler production as the number one agricultural commodity and the largest contributor to the agricultural market in the state, it is imperative that producers be aware of their environmental impact and utilize phytases in their diets [71]. The Chesapeake Bay Total Maximum Daily Load states the necessary reductions of nitrogen and P that West Virginia must comply with in order to reduce run-off to the Bay. More avenues of P reduction are still necessary to decrease West Virginia's contribution to environmental pollution. Therefore, the research and development of phytases with increased efficacy are needed.

There are also benefits in exploring options for a more heat-stable enzyme that can withstand the pelleting process. Furthermore, it is imperative to continue searching for phytase enzymes that retain activity at different pH levels to accommodate for the ranges in the poultry digestive tracts. Finally, a study conducted to evaluate the temperature or retention time at which Lys levels and corresponding amino acid densities decrease would be optimal for the knowledge of feed manufacturers. Not only is it beneficial to evaluate the AA densities, but it would be beneficial for the feed manufacture industry to determine at which temperature and retention time it takes to reduce pathogen loads while maintaining optimal AA levels and reducing detriments to bird performance.

References

1. Abdollahi, M. R., V. Ravindran, and B. Svihus. 2013. Pelleting of broiler diets: An overview with emphasis on pellet quality and nutritional value. Anim. Feed. Sci. Tech. 179:1-23.
2. Adedokun, S. A. and O. Adeola. 2013. Calcium and phosphorus digestibility: Metabolic limits. Journal of Applied Poultry Research 22:600-608.
3. Adeola O and Cowieson AJ, Board-invited review: opportunities and challenges in using exogenous enzymes to improve nonruminant animal production. J Anim Sci 89:3189–3218 (2011).
4. Ayres, V.E., M.E. Jackson, S. A Cantley, S.J. Rochell, C.D. Crumpacker, D.T. Lee, B.C. Bodle, W.J. Pacheco, M.S. Rueda, C.A. Bailey, K. Gardner, T.P. Boltz, J.S. Moritz, Plus® on 21-day broiler performance and tibia mineralization, Journal of Applied Poultry Research, Volume 30, Issue 4, 2021, 100210, ISSN 1056-6171
5. Barua, M et al. Standardized ileal amino acid digestibility of protein sources for broiler chickens is influenced by the feed form. Poultry science vol. 99,12 (2020): 6925-6934. doi:10.1016/j.psj.2020.09.040
6. Bedford, M. R., C. L. Walk, and H. V. Masey O'Neill. 2016. Assessing measurements in feed enzyme research: Phytase evaluations in broilers. J. Appl. Poult. Res. 25(2):305–314
7. Bedford, M.R., Exogenous enzymes in monogastric nutrition—Their current value and future benefits, Anim. Feed Sci. Technol., 86 (1–2) (2000), pp. 1-13
8. Behnke, K. C. 1994. Factors affecting pelleting quality. Pages 44-54 in Proc. Maryland Nutr. Conf., College Park, MD. Maryland Feed Ind. Counc. and Univ. Maryland, College Park

9. Benevenga, N. J, Blemings, K. P., Unique Aspects of Lysine Nutrition and Metabolism, The Journal of Nutrition, Volume 137, Issue 6, June 2007, Pages 1610S–1615S.

10. Bergeron, A.N., Boney, J.W., Moritz, J.S., The Effects of Diet Formulation and Thermal Processes Associated With Pelleting on 18-day Broiler Performance and Digestible Amino Acid Concentration, Journal of Applied Poultry Research, Volume 27, Issue 4, 2018, Pages 540-549, ISSN 1056-6171.

11. Bohn, Lisbeth et al. "Phytate: impact on environment and human nutrition. A challenge for molecular breeding." *Journal of Zhejiang University. Science. B* vol. 9,3 (2008): 165-91. doi:10.1631/jzus.B0710640

12. Boltz, T.P., J.W. Boney, C. Shen, J. Jaczynski, J.S. Moritz, The Effect of Standard Pelleting and More Thermally Aggressive Pelleting Utilizing a Hygieniser on Feed Manufacture and Reduction of Enterococcus faecium, a Salmonella Surrogate, Journal of Applied Poultry Research, Volume 28, Issue 4, 2019, Pages 1226-1233, ISSN 1056-6171

13. Boney, J.W., J. Jaczynski, J.L. Weidhaas, A.N. Bergeron, J.S. Moritz, The effects of steam conditioning and antimicrobial inclusion on feed manufacturing and inactivation of Enterococcus faecium, a Salmonella surrogate, Journal of Applied Poultry Research, Volume 27, Issue 4, 2018, Pages 472-482, ISSN 1056-6171

14. Boney, J.W., J.S. Moritz, Phytase dose effects in practically formulated diets that vary in ingredient composition on feed manufacturing and broiler performance, Journal of Applied Poultry Research, Volume 26, Issue 2, 2017, Pages 273-285, ISSN 1056-6171.

15. Boney, J.W., J.S. Moritz, The effects of Spirulina algae inclusion and conditioning temperature on feed manufacture, pellet quality, and true amino acid digestibility, Animal Feed Science and Technology, Volume 224, 2017, Pages 20-29, ISSN 0377-8401

16. Boney, John William. Evaluating Steam Conditioning Manipulations and Grain Expressed Enzymes Effects on Feed Manufacturing Metrics, Feed Hygiene, Broiler Performance, and Bone Mineralization. Order No. 10643065 West Virginia University, 2017. Ann Arbor: ProQuest. Web. 22 Mar. 2022.

17. Boroojeni, F.G., B. Svihus, H.G. von Reichenbach, J. Zentek, The effects of hydrothermal processing on feed hygiene, nutrient availability, intestinal microbiota and morphology in poultry-A review, Anim. Feed Sci. Technol., 220 (2016), pp. 187-215

18. Bowen, K. M., Improving Broiler Performance and Digestibility Through Feed Enzymes and Production Rate Aids Utilized in Pelleting. 2021. West Virginia University. MS Thesis. *Research Repository*.

19. Buchanan, N. & Moritz, J. (2009). Main effects and interactions of varying formulation protein, fiber, and moisture on feed manufacture and pellet quality. Poultry Science Association J. Appl. Poult. Res. 18. 274-283. 10.3382/japr.2008-00089.

20. Calet, C. 1965. The relative value of pellets versus mash and grain in poultry nutrition. World Poultry Sci. J. 21:23-52.

21. Campasino, T. York, C. Wyatt, M.R. Bedford, W.A. Dozier, Effect of increasing supplemental phytase concentration in diets fed to Hubbard × Cobb 500 male broilers from 1 to 42 days of age1, Journal of Applied Poultry Research, Volume 23, Issue 4, 2014, Pages 705-714, ISSN 1056-6171.

22. Cheftel J.C. Nutritional effects of extrusion cooking. Food Chem. 1986;20:263–283

23. Chen, R., Xue, G., Chen, P. et al. Transgenic maize plants expressing a fungal phytase gene. Transgenic Res 17, 633–643 (2008). https://doi.org/10.1007/s11248-007-9138-3

24. Cowieson A.J., Ravindran V. Effect of exogenous enzymes in maize-based diets varying in nutrient density for young broilers: growth performance and digestibility of energy: minerals and amino acids. Br. Poult. Sci. 2008;49:37–44.

25. Cowieson, A.J, T. Acamovic, M.R. Bedford. Phytic acid and phytase: Implications for protein utilization by poultry. Poult. Sci., 85 (2006), pp. 878-885, 16673766.

26. Cutlip, S. E., J. M. Hott, N. P. Buchanan, A. L. Rack, J. D. Latshaw, and J. S. Moritz. 2008. The effect of steam-conditioning practices on pellet quality and growing broiler nutritional value. J Appl Poult Res. 17:249-261.

27. Davies, R. H, and C. Wray. 1997. Distribution of Salmonella contamination in ten animal feed mills. Vet. Med. 57:159-169.

28. Dersjant-Li, Y., Awati, A., Schulze, H. and Partridge, G. (2015), Phytase in non-ruminant animal nutrition: a critical review on phytase activities in the gastrointestinal tract and influencing factors. J. Sci. Food Agric., 95: 878-896. https://doi.org/10.1002/jsfa.6998

29. Dilger, R.N., E.M. Onyango, J.S. Sands, O. Adeola, Evaluation of Microbial Phytase in Broiler Diets1, Poultry Science, Volume 83, Issue 6, 2004, Pages 962-970, ISSN 0032-5791

30. Drezner, M. K. 2002. Phosphorus homeostasis and related disorders. Pages 321-338 in Principles of Bone Biology. Vol. 1. J.

31. Edwards, D.R., T.C. Daniel, Environmental impacts of on-farm poultry waste disposal — A review, Bioresource Technology, Volume 41, Issue 1, 1992, Pages 9-33, ISSN 0960-8524

32. Engelen, A. J., F. C. van der Heeft, P. H. G. Randsdorp, W. A. C. Somers, J. Schaefer, and B. J. C. van der Vat. 2001. Determination of phytase activity in feed by a

colorimetric enzymatic method: Collaboration interlaboratory study. J. AOAC Int. 84:629–633.

33. Erdman, J. W. 1979. Oilseed phytates: Nutritional implications. J. Am. Oil Chem. Soc. 56:736–741.
34. Farshad Goodarzi Boroojeni, Birger Svihus, Heinrich Graf von Reichenbach, Jürgen Zentek, The effects of hydrothermal processing on feed hygiene, nutrient availability, intestinal microbiota and morphology in poultry—A review, Animal Feed Science and Technology, Volume 220, 2016, Pages 187-215, ISSN 0377-8401.
35. Fontaine, J., U. Zimmer, P. J. Moughan, and S. M. Rutherfurd. 2007. Effect of heat damage in an autoclave on the reactive Lys contents of soy products and corn distillers dried grains with solubles. Use of the results to check on Lys damage in common qualities of these ingredients. Journal of Agricultural and Food Chemistry. 55:10737-10743.
36. González-Vega, J. C., B. G. Kim, J. K. Htoo, A. Lemme, and H. H. Stein. 2011. Amino acid digestibility in heated soybean meal fed to growing pigs. J Anim Sci. 89:3617-3625. 19. Juzaitis-Boelter, C. P., A.P. Benson, M. U. Ahammad, M. K. Jones, J. Ferrel, and A. J
37. Gous, R. M. 1998. Making progress in the nutrition of broilers. Poult. Sci. 77:111–117.
38. Holsheimer, J.P. and E.W. Ruesink, 1998. Processing losses, performance, carcass composition, yield and financial return of dietary energy and lysine levels in starter and finisher diet fed to broiler. Poult. Sci., 77: 806-815.
39. Homan, V.B., J.W. Boney, J.S. Moritz, Effects of steam conditioning temperatures on commercial phytases and subsequent broiler performance and tibia mineralization. Appl. Anim. Sci., 35 (2019), pp. 298-303

40. Hong CY, Cheng KJ, Tseng TH, Wang CS, Liu LF, Yu SM. Production of two highly active bacterial phytases with broad pH optima in germinated transgenic rice seeds. Transgenic Res. 2004 Feb;13(1):29-39. doi: 10.1023/b:trag.0000017158.96765.67. PMID: 15070073.

41. Hott, J.M., N.P. Buchanan, S.E. Cutlip, J.S. Moritz, The Effect of Moisture Addition with a Mold Inhibitor on Pellet Quality, Feed Manufacture, and Broiler Performance1, Journal of Applied Poultry Research, Volume 17, Issue 2, 2008, Pages 262-271, ISSN 1056-6171.

42. Ishii, Toshiyuki et al. "Dietary Supplementation with Lysine and Threonine Modulates the Performance and Plasma Metabolites of Broiler Chicken." The journal of poultry science vol. 56,3 (2019): 204-211. doi:10.2141/jpsa.0180104

43. Jensen, L.S, L.H. Merrill, C.V. Reddy, J. Mc-Ginnis, Observations on eating patterns and rate of food passage of birds fed pelleted and unpelleted diets, Poult. Sci., 41 (1962), pp. 1414-1419

44. Kidd. M.T., Kerr, B.J, Halpin K.M., McWard, G.W., Quarles, C.L. Lysine Levels in Starter and Grower-Finisher Diets Affect Broiler Performance and Carcass Traits, Journal of Applied Poultry Research, Volume 7, Issue 4, 1998, Pages 351-358, ISSN 1056-6171,

45. Kim, Taewan et al. "Shifting the pH profile of Aspergillus niger PhyA phytase to match the stomach pH enhances its effectiveness as an animal feed additive." Applied and environmental microbiology vol. 72,6 (2006): 4397-403. doi:10.1128/AEM.02612-05

46. Kumar, V., A. Miasnikov, J. S. Sands, and P. H. Simmins. 2003. In vitro activities of three phytases under different pH and protease challenges. APSA Conf. P. 164-164

47. Leclercq B. Specific effects of lysine on broiler production: domparison with threonine and valine. *Poultry Science*, 77: 118-123. 1998.

48. Leeson, S. and J. D. Summers. 2001. Nutrition of the Chicken. 4th Edition. University Books. Guelph, Ontario Canada

49. Liu, Huichao & Ding, Peng & Tong, Yueyue & He, Xi & Yin, Yulong & Zhang, Haihan & Song, Zehe. (2021). Metabolomic analysis of the egg yolk during the embryonic development of broilers. Poultry Science. 100. 10.1016/j.psj.2021.01.036.

50. Loar R.E. II, K.G.S. Wamsley, A. Evans, J.S. Moritz, A. Corzo. Effects of varying conditioning temperature and mixer-added fat on feed manufacturing efficiency, 28- to 42-day broiler performance, early skeletal effect, and true amino acid digestibility1. J Appl. Poult. Res., 23 (2014), pp. 444-455

51. Loop, S.A., K.G.S. Lilly, L.K. Shires, C.K. Gehring, K.R. Beaman, M.E. Persia, J.S. Moritz, The phytase analytical activity of pelleted diets may not adequately describe efficacy in the bird, Journal of Applied Poultry Research, Volume 21, Issue 3, 2012, Pages 492-501, ISSN 1056-6171

52. Ludikhuyse L., Van Loey A., Indrawati S.C., Hendrickx M. Effects of combined pressure and temperature on enzymes related to quality of fruits and vegetables: from kinetic information to process engineering aspects. *Crit. Rev. Food Sci. Nutr.* 2003;43:527–586.

53. Maga, J. A. 1982. Phytate: Its chemistry, occurrence, food interactions, nutritional significance, and methods of analysis. J. Agric. Food Chem. 30:1–9

54. Menezes-Blackburn D, Gabler S, Greiner R. Performance of Seven Commercial Phytases in an in Vitro Simulation of Poultry Digestive Tract. J Agric Food Chem. 2015 Jul 15;63(27):6142-9. doi: 10.1021/acs.jafc.5b01996. Epub 2015 Jul 2. PMID: 26111064.

55. Moreno, F. J., E. Molina, A. Olano, and R. López-Fandiño. 2003. High-pressure effects on Maillard reaction between glucose and Lys. Journal of Agricultural and Food Chemistry. 51:394-400.

56. Moritz, J.S., R.S. Beyer, K.J. Wilson, K.R. Cramer. Effect of moisture addition at the mixer to a corn-soybean-based diet on broiler performance. J. Appl. Poult. Res., 10 (2001), pp. 347-353

57. Moughan, P. J., Rutherfurd, S. M., Available Lysine in Foods: A Brief Historical Overview, *Journal of AOAC INTERNATIONAL*, Volume 91, Issue 4, 1 July 2008, Pages 901–906.

58. Nelson, T.S., T.R. Shieh, R.J. Wodzinski, J.H. Ware, The Availability of Phytate Phosphorus in Soybean Meal Before and After Treatment With a Mold Phytase, Poultry Science, Volume 47, Issue 6, 1968, Pages 1842-1848, ISSN 0032-5791.

59. Parsons, C. M. 1996. Digestible amino acids for poultry and swine. Anim. Feed. Sci. Tech. 59:147-153.

60. Paullauf, J., D. Hohler, G. Rimbach, and H. Neusser. 1992. Influence of an addition of microbial phytase to a corn-soy diet on the apparent absorption of P and calcium in the piglet. J. anim. Physiol. Anim. Nutr. 67:30-40.

61. Peisker, M. 2006. Feed Processing- Impacts on Nutritive Value and Hygienic Status in Broiler Feeds. Aust. Poult. Sci. Symp. 18.

62. Properties of Amino Acids. 30 July 2020, https://chem.libretexts.org/@go/page/241691.

63. Sara I.F.S Martins, Wim M.F Jongen, Martinus A.J.S van Boekel, A review of Maillard reaction in food and implications to kinetic modelling, Trends in Food Science & Technology, Volume 11, Issues 9–10, 2000, Pages 364-373, ISSN 0924-2244.

64. Sell, J.L., & Thompson, O.J. (1965) The effects of ration pelleting and level of fat on the efficiency of nutrient utilisation by the chicken, British Poultry Science, 6:4, 345-354

65. Selle, P. H., and V. Ravindran. 2007. Microbial phytase in poultry nutrition. Anim. Feed Sci. Technol. 135:1-41

66. Shirley, R. B. and H. M. Edwards. 2003. Graded levels of phytase past industry standards improves broiler performance. Poultry Science. 82:671-680.

67. Shivange, A.V., Hoeffken H. W., Haefner, S., and Schwaneberg, U. Protein consensus-based surface engineering (ProCoS): a computer-assisted method for directed protein evolution. BioTechniques. 2016 61:6, 305-314

68. Soni, S.K., Khire, J.M. Production and partial characterization of two types of phytase from Aspergillus niger NCIM 563 under submerged fermentation conditions. World J Microbiol Biotechnol 23, 1585–1593 (2007).

69. Thomas, M., and A.F.B. van der Poel. 1996. Physical quality of pelleted animal feed Criteria for pellet quality. Animal Feed Sci. and Tech. 61:89-112.

70. Tumuluru, J.S., High moisture corn stover pelleting in a flat die pellet mill fitted with a 6 mm die: physical properties and specific energy consumption. Energy Sci Eng, 3: 327-341. 2015 https://doi.org/10.1002/ese3.74

71. USDA. September 04, 2019. 2019 Farm Cash Receipts in West Virginia. [Press Release]

72. Veum, T. L. 2010. Phosphorus and Calcium Utilization and Requirements in Farm Animals. D. M. S. S. Vitti and E. Kebreab, ed. CAB International, Oxfordshire, UK.

73. Vohra A, Satyanarayana T (2003) Phytases: microbial sources, production, purification and potential biotechnological applications. Crit Rev Biotechnol 23:29–60

74. Yan, F., A. Fritts, P.W. Waldrup, Evaluation of modified dietary phosphorus levels with and without phytase supplementation on live performance and excreta phosphorus concentration in broiler diets 2. Modified early phosphorus levels. J. Appl. Poult. Res., 13 (2004), pp. 394-4

CHAPTER TWO

The Effects of Hygienic Pelleting in Diets that Differ in Amino Acid Density on Ross 708 Broiler Performance, Amino Acid Digestibility, and Requirement

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ABSTRACT

To reduce pathogenic bacteria such as *Salmonella* in feed and ensure a high-quality pellet, diets for broiler and turkey breeders may be pelleted using high conditioning temperatures and times. However, increased conditioning temperature and time may alter amino acid conformation, decrease digestibility, and subsequent bird performance. Increasing formulation density of amino acids may be necessary to maintain performance. The objective of this study was to evaluate high conditioning temperature and exposure time within the pelleting process using a hygieniser (Hygienic Pelleting) in diets that vary in digestible Lys and corresponding amino acid ratio on Ross 708 starter broiler performance, amino acid digestibility, and requirement. A 5 x 2 factorial arrangement of treatments with 5 levels of digestible Lys (-20%, -10%, 2019 Ross Broiler Starter Nutrition recommendation, +10%, and +20%) and 2 degrees of pelleting (Standard and Hygienic) was utilized in a randomized complete block design. Each

treatment was fed to 12 replicate pens of 10 male broiler Hubbard x Ross 708 chicks for a 21-day period. On the morning following day 7, 14 and 21, birds and feed were weighed by pen for performance calculations and ileal contents were collected on d22 for AA digestibility calculations. Standard Pelleting and Hygienic Pelleting demonstrated a 1.19 and 1.37% Lys requirement, respectively, using the broken-line regression model. Performance data and the broken-line regression model suggest an approximate 10% increase in Lys and corresponding amino acid ratio relative to 2019 Ross Broiler Starter Nutrition Specifications for Hygienic Pelleting to provide optimal live weight gain. Hygienic Pelleting decreased amino acid digestibility and increased subsequent FCR when diets were formulated to decreased amino acid density.

Keywords: Lysine, Amino Acid Digestibility, Hygieniser, Thermal Processing, Feed Efficiency

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DESCRIPTION OF THE PROBLEM

High value is placed on broiler breeders due to the large financial, time, and resource investment. Precautionary steps can be taken during the feed manufacture process to support broiler breeder health and safety, ultimately protecting the production investment. One of these steps include increased conditioning temperature and time to reduce pathogenic bacteria loads that may be associated with feed or feed ingredients. The use of a hygieniser (jacketed heating system used to maintain feed at an elevated temperature) may facilitate protection. Studies have shown physical quality and pathogen reduction benefits to pelleting feed with increased conditioning temperature and retention time. Increased conditioning temperatures have been shown to improve pellet quality and durability [7]. Boltz, et al. found a 3- and 4-log reduction of

Enterococcus faecium (a *Salmonella* surrogate) when evaluating the differences between two pelleting techniques: standard pelleting of 70°C conditioning for 15s without the hygieniser and a more thermally aggressive pelleting of 80°C conditioning for 30s with a 45s retention time in the hygieniser [6].

Conversely, due to these atypical thermal processing conditions, heat-labile amino acids (**AA**) such as Lysine (**Lys**) and Cysteine may become degraded or bound. Hygienic pelleting provides the necessary conditions to induce a Maillard browning reaction that would render Lys, the second limiting AA in poultry diets, unavailable. This reaction is initiated by heat and results in the interaction between a carbonyl group of a reducing sugar and an amino compound, followed by rearrangement and production of an amadori product [5]. Fontaine and coauthors [13] noted that when heated in an autoclave at 135°C, soybean meal commonly experienced 10-20% Lys damage, and when overheated could easily lose up to 67% reactive Lys. Reactive Lys is defined as lysine that has not undergone the Maillard reaction, is metabolizable, and can be described as unmodified lysine which possesses a free side chain amino group and can be either free or protein bound [28]. If nutritionists expect AA loss due to feed pelleting practices, then poultry diets may be over-formulated in AA pre-pelleting. However, formulating diets above requirement for expensive nutrients, such as AA, would be economically unfavorable. Therefore, the over-formulation should be based on comprehensive data that explores these specific variables.

The combination of the potential for AA conformational change, the expense of AA, and the tendency of using high conditioning temperature and time as a safeguard in hygienic pelleting, necessitates an understanding of AA formulation density that maintains broiler breeder profitability. The objective of this study was to determine the effects of Hygienic Pelleting

versus Standard Pelleting using varying Lys levels and corresponding AA ratios on Ross 708 broiler performance, AA digestibility, and requirement. This study provides insight to the degree of over-formulation necessary to maintain breeder performance with hygienic feed.

MATERIALS AND METHODS

Diet Formulation and Batching

All feed was manufactured at the West Virginia University pilot feed mill in Morgantown, West Virginia. A 5 (Formulated Lys Level) x 2 (Pelleting Treatment) factorial treatment arrangement was used. Two basal diets, 20% below Ross Levels of Lys and 20% above Ross Levels of Lys, were formulated using the 2019 Ross Broiler Starter Nutrition Specifications for target live weight of ≤ 1.60 kg, 0-10 days, as a nutrient reference for broiler chicks (**Table 1**). Digestible (**dig**) Lys to amino acid ratios were adjusted as per the 2019 Ross Broiler Starter Nutrition Specifications.

Master batches of the -20% diet (1406.14 kg) and +20% diet (1406.14 kg) were mixed in a one-ton vertical ribbon mixer. Studies have shown that the addition of fat reduced heat caused by friction at the pellet die. Added fat aids in overall lubrication of the die thus requiring less energy for the feed to be extruded through the die [24]. In order to maintain consistency throughout the pelleting process, 0.79% of soybean oil (total oil content of the deficient master batch) was added at the mixer. Diets requiring more than the minimum amount of soybean oil had the fat added post crumbling. A 50/50 titration of the -20% and +20% diet, respectively, was used to develop the base diet, henceforth called the Ross level diet. A 75/25 blend was used to develop the -10% below Ross levels diet (-10%) and a 25/75 titration blend was used to develop

the +10% above Ross levels diet (+10%). Each of these five dig Lys levels amounting to 544.31kg were manufactured under one of two conditioning methods of Standard Pelleting or Hygienic Pelleting.

Feed manufacture

On the day of feed manufacture, a high-fiber ruminant diet formulation was utilized to warm-up the feed mill equipment. Next, 544 kg of each dig Lys level was sent to the surge bin above the pelleting equipment stack. One half of this volume was pelleted using Standard Pelleting (77℃ in the conditioner for 30 seconds) and then the other half was pelleted in sequence using Hygienic Pelleting (88℃ in the conditioner for 60 seconds and a 6-minute retention time in the activated hygieniser). Post conditioning/hygienization, all treatments were extruded through a 4.76 x 38 mm pellet die, cooled, and crumbled. If the diet required fat over the mixer added 0.79%, additional fat was added post-crumbling. During processing, mash samples, hot-pellet temperature, pellet samples, crumble samples (after fat-addition), and pellet samples for Pellet Durability Index (**PDI**) were taken to provide descriptive feed manufacture data (**Table 2**). Crumbled feed was put into burlap bags and stored in a 4.6 x 2.4 m insulated storage room equipped with a dehumidifier (Hisense 50-Pint 2-Speed Dehumidifier; Hinsese, Suanee, GA) to equilibrate moisture content among treatments prior to feeding. Due to the variability between conditioning treatments, it was imperative that moisture level be standardized. All diets were analyzed for dry matter percentage (**DM%**) to ensure equal moisture content in the feed [20].

Sampling

Composite samples of pellets, crumbles, and mash were formed using a sample splitter. Samples were kept in an 18℃ freezer until they were ready to be analyzed. Pellet Durability Index was determined 24 hours after manufacture using a New Holmen Pellet Tester (**NHPT**) where 100g of pelleted samples were subjected to air flow within a perforated chamber for 30 seconds. Particle size determination of crumble samples was performed to ensure standardized feed form [8, 10].

Live Bird Performance

A total of 1,200, 1-day old, male Hubbard x Ross 708 broiler chicks were obtained from a local commercial hatchery (Longenecker's Hatchery Elizabethtown, PA). On day 1 (d1), birds were weighed and separated into 120 groups of ten chicks to create uniform initial pen weights. Treatments were assigned using a randomized complete block design. A block consisted of ten adjacent pens and a total of twelve blocks were utilized. Chicks were housed in a raised wire cage system in a cross-ventilated, negative pressure room for 21 days. Three identical rooms were utilized with 40 pens (four blocks) per room. Standard rearing conditions were used during the entirety of the study. Room temperature for the 1-day-old chicks was set at 32°C, and gradually decreased to 29°C for the second week and 26°C for the third week of the study, to create optimal rearing conditions. Feed was placed in external feed troughs and water was supplied through a nipple drinker system; both feed and water were provided for ad libitum consumption. From d1 to 6, birds were exposed to 24h light, and after d6, the hours of light were decreased gradually until 6h dark was reached on d21. Mortalities were recorded and mortality weights were taken throughout the entirety of the study. Pen weights were taken on the morning after d7, d14, and d21 for calculations of live weight gain (**LWG**), feed intake (**FI**), mortality

adjusted feed conversion ratio (**FCR**), individual bird weight (**IBW**) and mortality percentage. All animals were reared according to protocols established by the West Virginia University Animal Care and Use Committee. (IACUC Protocol #1602000612_R1)

Amino Acid Digestibility

Birds were exposed to 6h of dark, followed by 6h of light to ensure that birds had a full GI tract when sampled. On the morning of d22, 6 birds per pen were randomly selected for digesta collection. The digestive tract from the Meckel's diverticulum to the ileo-cecal junction was first removed from the bird. De-ionized water was pushed through the lower half of this section to remove digesta. A pooled sample was taken from each pen. Digesta samples underwent lyophilization. The samples were then ground and analyzed for amino acid and titanium dioxide content using AOAC Official Method 994.12 [3, 27]. These data were used to calculate Apparent Ileal Digestibility (**AID**) and Digestible Amino Acid Concentration (**DAAC**) [5].

Apparent ileal digestibility value was found using the following formula:

$$AID\,(\%) = \left[1 - \left(\frac{TD_i}{TD_0}\right) x \left(\frac{N_o}{N_i}\right)\right] x100$$

Digestible amino acid concentration was calculated using the following formula:

$$DAAC = \left(\frac{AID}{100}\right) x\, TAA$$

Where Td_i represents the concentration of TiO_2 in the diet in grams per kilogram of DM; Td_o represents the concentration of TiO_2 in the ileal digesta in grams per kilogram of DM output; Ni represents the concentration of nitrogen or AA in the diet in grams per kilogram DM; and No represents the concentration of nitrogen or AA in ileal digesta in

grams per kilogram of DM. The TAA value represents the total amino acid concentration found in the diet [2, 5].

Statistical Analysis

A 5 (Formulated Lys Level) by two (Pelleting Treatment) factorial arrangement in a randomized complete block design was utilized with one pen of 10 broilers as the experimental unit. Data were analyzed using GLM procedure of SAS [31] considering pen location within the room as the blocking criterion. Significance was set at $P < 0.05$ and tendency at $P < 0.10$. Significant Analysis of Variance (**ANOVA**) results were further analyzed using the Fisher's Least Significant Difference (**LSD**) test and letter superscripts to denote differences among treatment means.

A Broken-Line Regression Model (**BLRM**) was used to determine dig Lys level requirement for optimal d0-21 LWG [29]. The use of SAS PROC NLIN has been described as a simple, quick, and efficient means of fitting the broken 1ine to growth data [30]. Therefore, the SAS PROC NLIN was used to fit a simple 2 straight-line, one-breakpoint model. Parameters were defined as the breakpoint x value (R), an asymptote for the first segment (L), and slopes for the two line segments (U). The parameters were as follows:

Parameters	Standard	Hygienic
L	0.830	0.851
U	-2853	-2853
R	1.28	1.28

RESULTS AND DISCUSSION

Feed Manufacture Results

Descriptive feed manufacture data are presented in **Table 2**. The increased conditioning temperature during Hygienic Pelleting decreased motor load. This was likely due to increased lubrication at the pellet die due to increased temperature and moisture level. Both Fairchild and Hott observed a decrease in pellet mill energy consumption when moisture was added to the diet [12, 15]. Hot pellet temperature increased an average of 8.06°C as conditioning temperature increased from Standard Pelleting to Hygienic Pelleting, as per design. On average, Hygienic Pellets had 4.1 percentage points higher durability than Standard Pellets. Studies have shown that increasing retention time during conditioning will increase PDI [11, 14]. Furthermore, as conditioning temperature increases, PDI and pellet quality has also been known to increase [11, 23, 32]. A study done by Cutlip et. al showed that increasing conditioning temperature increased PDI (P = 0.0001) and modified PDI (P=0.0001) [8]. Variation among treatment particle size did not exceed 200 microns, likely having a low confounding effect on bird performance.

Live Bird Performance Results

Live bird performance results are shown in **Table 3.** Formulated Lysine Level was significant for d0-14 FCR, LWG, and IBW (P<0.0001, P<0.0001, and P=0.0005 respectively). As dig Lys levels and corresponding AA ratios increased, FCR decreased and both LWG and IBW increased. A study done by Kidd and cohorts supports these results. They found that increasing dietary Lys in a crumbled starter diet from 95 to 115% of NRC improved body weight and feed to gain ratios, concluding that feeding broilers above NRC recommendations improved broiler performance [18]. Increasing Lys levels over National Research Council (**NRC**) levels

was reported to improve FCR, breast meat yield, and BWG with low abdominal fat pad weight in a study performed by Leclercq [21].

Significant interactions between Formulated Lys Level and Pelleting Treatment were observed for d0-21 FCR, LWG, and IBW (P=0.0001, 0.0443, and 0.0431, respectively). Hygienic Pelleted treatments necessitated higher levels of dig Lys and corresponding AA ratios to maximize gain and minimize FCR. These data suggest that a 10% increase in dig Lys and corresponding AA ratios relative to 2019 Ross Broiler Starter Nutrition Specifications are necessary for Hygienic Pelleted treatments to achieve a LWG similar to the Ross Level Standard Pelleting diet. It should be noted that these data encompass d0-21 performance and that the 2019 Ross Broiler Starter Nutrition Specifications referenced were for d0-10 nutrition.

Increased AA density recommendation are supported with the BLRM. **Figure 1** shows that dig Lys requirement was met at 1.19% for optimal LWG in broilers with Standard Pelleting. This is below the Ross Lys level recommendation, 1.28% for d0-10. However, dig Lys requirement increased to 1.37% with Hygienic Pelleting (**Figure 2**). It can be assumed that a 9% overage from 2019 Ross Broiler Starter Nutrition Specifications is needed to accommodate for thermal processing nutrient detriments during Hygienic Pelleting.

Amino Acid Digestibility

Due to the performance results and BLRM, only six of the ten treatments were selected to determine AA digestibility. The most extreme treatments, -20% and +20%, were omitted. Apparent ileal digestibility data for the -10%, Ross level, and +10% diet at both Standard and Hygienic Pelleting are reported in **Table 4**. Significant interactions between Formulated Lys Level and Pelleting Treatment were observed for all AA (P<0.0001). A decrease in digestibility

was observed for the -10% Hygienic Pelleting diet when compared to the -10% Standard Pelleting diet (P<0.05). This finding is supported with a study done by Loar et. al, who stated that conditioning corn and SBM-based diets above 74°C decreased AA digestibility [23]. A study done by Bergeron reported an interaction between low AA density diets with a bakery by-product inclusion and processing effects with 3 degrees of thermal processing (unprocessed mash, pellet, double pellet). The interaction affected the DAAC of Lys and cysteine (P < 0.05). Digestible Lys and cysteine concentrations decreased when the diet was pelleted and double pelleted [5]. In the current study, as dig Lys level increased to the Ross level and +10%, digestibility between pelleting treatments were statistically similar (P<0.0001). An illustration of the interaction for AID of Lys can be found in **Figure 3**. This interaction aligns with d0-21 FCR data expressed using the same treatments and is shown in **Figure 4**. It can be suggested that birds were required to consume numerically more feed for the -10% Hygienic Pelleting diet to compensate for the detriment of dig Lys and decreased amino acid densities caused by increased thermal processing and, regardless, gained numerically less weight (P<0.05). This in turn, increased FCR by 10 points when compared to the -10% Standard Pelleting diet. Decreased AA density seemed to exacerbate the nutrient detriment caused by Hygienic Pelleting. Hygienic Pelleting decreased amino acid digestibility and increased subsequent FCR when diets were formulated to decreased amino acid density.

An increase in the height and width of the villi in poultry intestines is closely related to an increase in digestive and absorptive function due to increased surface area [4]. The reverse is also true. A decrease in either villus height or crypt depth can lead to a reduction in nutrient absorption [19]. Studies have also shown that increasing or decreasing nutrients within the diet can affect villi size. Increasing glutamine by 0.8% improved the growth of the duodenum and led

to an increased villus height [9]. A study done by Lisnahan and cohorts showed that the supplementation of 0.17% L-Tryptophan and 0.68% L-Threonine produced increased villi height, crypt depth, and villi width during the starter phase in birds [22]. Conversely, Abbasi found that a decrease in dietary crude protein led to significantly lowered jejunal villi height and shallowed crypt depth in broilers [1]. In the current study, we speculate that decreased AA density coupled with Hygienic Pelleting in the -10% diet could have decreased villi height, crypt depth, or villi width which led to a decrease in AA digestibility.

The DAAC data are presented in **Table 5.** An interaction between Formulated Lys Level and Pelleting Treatment was observed for all AA ($P<0.05$). In particular, Lys as well as the branched chain AA had decreased DAAC for -10% and +10% Hygienic Pellets compared to the Standard Pellets but not the Ross level. The -10% and Ross level DAAC data align with the AID results. Perhaps the AA deficiency caused by both the reduced formulation density of -10% and nutrient detriment of Hygienic Pelleting may have decreased villus growth which reduced DAAC. Increasing AA density to Ross level may have alleviated the reduced villus growth and improved absorption. However, when increasing AA density to +10%, the increased formulation density combined with nutrient detriment of Hygienic Pelleting demonstrated a DAAC decrease relative to Standard Pellets. This observation was likely associated with nutrient detriments of Hygienic Pelleting. The decrease in DAAC at +10% with Hygienic Pellets could be caused by Maillard reaction products. Advanced glycation end-products (**AGEs**) are considered the final glycated products of the Maillard reaction. Glycation can cause the malformation and malfunction of affected proteins [33]. These small proteins when oxidized further and following cross-linking reactions, may form larger structures. The formation of large glycated proteins blocks the activity of proteasomes and makes them resistant to degradation leading to the

accumulation of AGEs in the cells and tissues, hindering tissue repair, and delaying turnover rate [16, 33, 34]. Because AA or peptides with free amino groups must also be present within the food to enable the Maillard reaction to take place and produce AGEs [17], it can be assumed that a diet with increased AA density processed at high temperatures would provide an optimal environment for an increased amount of AGEs. Perhaps Hygienic Pelleting with diets that contain increased dig Lys and corresponding AA ratios produced increased glycation end-products which then accumulated along the brush border thereby decreasing digestibility.

The previous speculation can be further supported when reviewing the techniques used to determine total amino acid concentrations. The process of determining Lys content in feed has been known to be problematic as High-Performance Liquid Chromatography (**HPLC**) techniques report total Lys amount as opposed to reactive versus non-reactive Lys. In the early stages of the Maillard reaction, the Amadori compounds formed due to processing can be hydrolyzed back to Lys in the presence of the strong acids utilized during HPLC acid hydrolysis. However, Lys bound to early stage Maillard reaction products will not be hydrolyzed back to Lys and will remain non-reactive within the digestive tract in the bird. Therefore, amino acid analysis using HPLC leads to an overestimation of digestible Lys content. In the advanced stages of the Maillard reaction, Lys is completely destroyed, not recoverable, and will not be hydrolyzed back to Lys with acid hydrolysis during AA analysis. Lys bound to advanced stage Maillard reaction products are not reported [26]. Therefore, it can be assumed that the decrease in DAAC observed for the +10% Hygienic diet compared to the +10% Standard diet may be attributed to increased advanced stage Maillard reaction products.

These experiments utilized a hygienic feed pelleting technique that was effective in decreasing AA digestibility and broiler performance. Through the use of graded levels of

digestible lysine and corresponding AA ratios, predictions for compensatory over formulation were derived.

CONCLUSIONS AND APPLICATIONS

1. Standard Pelleting and Hygienic Pelleting demonstrated a 1.19 and 1.37% Lys requirement, respectively, using the broken-line regression model.
2. Performance data and the broken-line regression model indicate an approximate 10% increase in Lys and corresponding amino acid ratio relative to 2019 Ross Broiler Starter Nutrition Specifications for Hygienic Pelleting to provide optimal live weight gain.
3. Hygienic Pelleting decreased amino acid digestibility and increased subsequent FCR when diets were formulated to decreased amino acid density.

REFERENCES

1. Abbasi, M. A.; Mahdavi, A. H.; Samie, A. H. and Jahanian, R. 2014. Effects of different levels of dietary crude protein and threonine on performance, humoral immune responses, and intestinal morphology of broiler chicks. Brazilian Journal of Poultry Science 16:35-44.
2. Adedokun, S.A., P. Jaynes, R.L. Payne, T.J. Applegate, Standardized Ileal amino acid digestibility of corn, corn distillers- Dried grains with solubles, wheat middlings, and bakery by-products in broilers and laying hens, Poult. Sci., 94. 2015, pp. 2480-2487
3. AOAC International, 2000, AOAC International, Official Methods of Analysis of the AOAC International, (17th ed.), AOAC International, Gaithersburg, MD. 2000
4. Awad W.A, Ghareeb K, Nitch S, Pasteiner S, Raheem S.A, Bohm J. Effect of dietary inclusion of probiotic, prebiotic and symbiotic on intestinal glucose absorption on broiler chickens. Int. J. Poult. Sci. 2008;7(7):688–691.
5. Bergeron A.N, J.W. Boney, J.S. Moritz, The Effects of Diet Formulation and Thermal Processes Associated With Pelleting on 18-day Broiler Performance and Digestible Amino Acid Concentration, Journal of Applied Poultry Research, Volume 27, Issue 4, 2018, Pages 540-549, ISSN 1056-6171
6. Boltz, T.P, J.W. Boney, C. Shen, J. Jaczynski, J.S. Moritz, The Effect of Standard Pelleting and More Thermally Aggressive Pelleting Utilizing a Hygieniser on Feed Manufacture and Reduction of Enterococcus faecium, a Salmonella Surrogate, Journal of Applied Poultry Research, Volume 28, Issue 4, 2019, Pages 1226-1233, ISSN 1056-6171

7. Boney, J.W., J.S. Moritz, The effects of Spirulina algae inclusion and conditioning temperature on feed manufacture, pellet quality, and true amino acid digestibility, Animal Feed Science and Technology, Volume 224, 2017, Pages 20-29, ISSN 0377-8401
8. Cutlip, S. E., J. M. Hott, N. P. Buchanan, A. L. Rack, J. D. Latshaw, and J. S. Moritz. 2008. The effect of steam-conditioning practices on pellet quality and growing broiler nutritional value. J Appl Poult Res. 17:249-261.
9. Dong, X.-Y., Yang, C.-F., Tang, S.-Q., Jiang, Q.-Y., & Zou, X.-T. 2010, June 21. Effect and Mechanism of Glutamine on Productive Performance and Egg Quality of Laying Hens. Asian-Australasian Journal of Animal Sciences. Asian Australasian Association of Animal Production Societies. https://doi.org/10.5713/ajas.2010.90611
10. Dozier, W.A., III, K.C. Behnke, C.K. Gehring, S.L. Branton. Effects of feed form on growth performance and processing yields of broiler chickens during a 42-day production period J. Appl. Poult. Res., 19. 2010, pp. 219-226
11. Fahrenholz, A. C. 2012. Evaluating factors affecting pellet durability and energy consumption in a pilot feed mill and comparing methods for evaluating pellet durability. PhD Diss. Kansas State University, Kansas.
12. Fairchild, F., and D. Greer. 1999. Pelleting with precise mixer moisture control. Feed Int. 20(8):32–36.
13. Fontaine, J., U. Zimmer, P. J. Moughan, and S. M. Rutherfurd. 2007. Effect of heat damage in an autoclave on the reactive Lys contents of soy products and corn distillers dried grains with solubles. Use of the results to check on Lys damage in common qualities of these ingredients. Journal of Agricultural and Food Chemistry. 55:10737-10743.

14. Gilpin, A.S., T.J. Herrman, K.C. Behnke, F.J. Fairchild, Feed moisture, retention time, and steam as quality and energy utilization determinants in the pelleting process, App. Eng. in Ag., 18, 2002, pp. 331-338
15. Hott, J.M, Buchanan, N.P., Cutlip, S.E., Moritz, J.S., The Effect of Moisture Addition with a Mold Inhibitor on Pellet Quality, Feed Manufacture, and Broiler Performance, Journal of Applied Poultry Research, Volume 17, Issue 2, 2008, Pages 262-271, ISSN 1056-6171
16. John, W., Lamb, E. The Maillard or browning reaction in diabetes. Eye 7, 230–237 (1993)
17. Kellow NJ, Coughlan MT. Effect of diet-derived advanced glycation end products on inflammation. Nutr Rev. 2015 Nov;73(11):737-59. doi: 10.1093/nutrit/nuv030. Epub 2015 Sep 15. PMID: 26377870.
18. Kidd. M.T., Kerr, B.J, Halpin K.M., McWard, G.W., Quarles, C.L. Lysine Levels in Starter and Grower-Finisher Diets Affect Broiler Performance and Carcass Traits, Journal of Applied Poultry Research, Volume 7, Issue 4, 1998, Pages 351-358, ISSN 1056-6171
19. Kuzmuk, K.N., Swanson, K.S., Tappenden, K. A., Schook, L.B., Fahey, G.C., Jr., Diet and Age Affect Intestinal Morphology and Large Bowel Fermentative End-Product Concentrations in Senior and Young Adult Dogs, The Journal of Nutrition, Volume 135, Issue 8, August 2005, Pages 1940–1945
20. Lamp, A.E. & Ward, N.E. & Wilson, J.W. & Moritz, J.S.. In vitro phytase activity post steam conditioning and pelleting may not consistently correlate with in vivo measurements among commercial phytases. Journal of Applied Poultry Research. 2020. 29. 10.1016/j.japr.2020.01.005.

21. Leclercq B. Lysine: Specific effects of lysine on broiler production: comparison with threonine and valine. Poult Sci. 1998 Jan;77,1 :118-23. doi: 10.1093/ps/77.1.118. PMID: 9469761.

22. Lisnahan, C. V., & Nahak, O. R., Growth performance and small intestinal morphology of native chickens after feed supplementation with tryptophan and threonine during the starter phase. 2020.Veterinary world, 13(12), 2765–2771.

23. Loar R.E. II, K.G.S. Wamsley, A. Evans, J.S. Moritz, A. Corzo. Effects of varying conditioning temperature and mixer-added fat on feed manufacturing efficiency, 28- to 42-day broiler performance, early skeletal effect, and true amino acid digestibility1. J Appl. Poult. Res., 23, 2014, pp. 444-455

24. Loar R.E., II & A. Corzo, Effects of feed formulation on feed manufacturing and pellet quality characteristics of poultry diets, World's Poultry Science Journal, 2011, 67:1, 19-28, DOI: 10.1017/S004393391100002X

25. Moreno, F. J., E. Molina, A. Olano, and R. López-Fandiño. 2003. High-pressure effects on Maillard reaction between glucose and Lys. Journal of Agricultural and Food Chemistry. 51:394-400.

26. Moughan P.J., Amino acid availability: aspects of chemical analysis and bioassay methodology. Nutr Res Rev. 2003 Dec;16(2):127-41. doi: 10.1079/NRR200365. PMID: 19087386.

27. Myers, W.D., P. A. Ludden, V. Nayigihugu, B. W. Hess, Technical Note: A procedure for the preparation and quantitative analysis of samples for titanium dioxide, *Journal of Animal Science*, Volume 82, Issue 1, January 2004, Pages 179–183

28. Parker-Norman, S., Using NIR to measure reactive lysine - the potential implications for the animal feed industry. 2017
29. Robbins K.R, A. M. Saxton, L. L. Southern, Estimation of nutrient requirements using broken-line regression analysis, *Journal of Animal Science,* Volume 84, Issue suppl_13, April 2006, Pages E155–E165
30. Robbins, Kelly R. A Method, Sas Program, and Example for Fitting the Broken-Line to Growth Data. Knoxville, Tenn: University of Tennessee Agricultural Experiment Station, 1986. Print.
31. SAS Institute. 2017. The SAS System for Windows 2017. Release 9.4 SAS Inst. Inc., Cary, NC.
32. Skoch, E. R., K. C. Behnke, C. W. Deyoe, and S. F. Binder, 1981.The effect of steam conditioning rate on the pelleting process. Anim. Feed Sci. Technol. 6:83
33. Teodorowicz, M., Hendriks, W. H., Wichers, H. J., & Savelkoul, H. 2018. Immunomodulation by Processed Animal Feed: The Role of Maillard Reaction Products and Advanced Glycation End-Products (AGEs). Frontiers in immunology, 9, 2088.
34. Vlassara H, Bucala R, Striker LJ. Pathogenic effects of advanced glycosylation: biochemical, biologic, and clinical implications for diabetes and aging. Lab Invest. (1994) 70:138–51.

TABLES AND FIGURES

Table 1. Starter Broiler Diet Formulations

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Ingredients	20% Low[1] (Lys-1.02)	20% High (Lys- 1.54)	Ingredients	20% Low (Lys-1.02)	20% High (Lys- 1.54)
Corn	64.03	37.63	**Calculated Analysis**		
Soybean meal (46%)	30.62	53.40	ME (kcal/lb)	1361.00	1361.00
Soybean oil	0.79	4.39	Crude Protein	18.70	27.64
Limestone	1.15	1.11	Dig Lys	1.02	1.54
Dicalcium Phosphate	1.95	1.80	Dig TSAA	0.78	1.14
DL – methionine	0.26	0.43	Dig Methionine	0.52	0.79
Lys – HCl	0.10	0.07	Dig Threonine	0.68	1.03
Vitamin/mineral Premix[2]	0.25	0.25	Dig Tryptophan	0.20	0.32
Salt	0.30	0.31	Calcium	0.96	0.96
Sodium bicarbonate	0.17	0.17	Non-phytate Phosphorus	0.48	0.48
Threonine	0.06	0.12	Sodium	0.18	0.18
Choline 70	0.10	0.10	Valine	0.81	1.19
Titanium Dioxide	0.20	0.20	**Lys to Amino Acid Ratios**		
Valine	0.01	0.02	Lys	100	100
			Methionine	51	51
			Methionine + Cystine	76	74
			Threonine	67	67
			Tryptophan	20	21
			Valine	79	77

[1]Nutrient requirements are based on 2019 Ross Broiler Starter Nutrition Specifications for target live weight of ≤ 1.60 kg, 0-10 days

[2]Supplied the following per kilogram of diet: manganese, 0.02%; 0.02%; zinc; 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 Nutrient Values

Pelleting Treatment[1]	Formulated Lys Level[2]	Thr	Cys	Met	Lys	Crude Protein
Standard	-10	0.72	0.35	0.69	1.23	20.42
	Ross Level	0.82	0.37	0.75	1.31	23.49
	10	0.99	0.42	0.81	1.56	25.07
Hygienic	-10	0.75	0.36	0.66	1.29	21.34
	Ross Level	0.81	0.38	0.71	1.35	23.38
	10	0.92	0.38	0.75	1.40	25.15

[1] Treatments are a combination of Lys level increases/decreases (in %) and feed mill processing treatments. Standard diets were conditioned at 76.67°C for 30 seconds and Hygienic diets were conditioned at 87.78°C for 60 seconds with a six-minute retention time in the hygieniser. The hygieniser was used to maintain temperature generated during steam conditioning

[2]Based off the live bird performance data and the broken-line regression model, the -20% and the +20% Formulated Lys Levels were omitted from testing

Table 3: Descriptive feed manufacture and physical quality data for dietary treatments

Pelleting Treatment[1]	Formulated Lys Level	Ambient Temperature (°C)	Ambient Humidity (%)	Motor Load[2] (%)	Steam Position[3] (Final %)	Hot Pellet Temperature[4] (°C)	NHPT[5]	Particle Size[6](microns)	Standard Deviation of Particle Size
Standard	-20	30.6	56	49	15	79.6	85.2	1627.76	2.00
	-10	30.6	54	48.5	15	80.2	88.1	1514.76	1.98
	Ross Levels	31.1	50	47.5	15	80.1	91.1	1550.72	1.98
	10	31.7	50	47	14	80.4	90.3	1575.31	2.01
	20	32.2	49	46.5	14	80.3	92.9	1583.17	1.98
Hygienic	-20	32.2	49	48	17	88	90.9	1557.09	1.98
	-10	32.2	49	46	17	88.2	92.5	1303.32	2.14
	Ross Levels	32.2	47	46.5	17	88.2	94.1	1680.39	2.01
	10	31.7	49	45	17	88.4	94.8	1460.87	2.02
	20	31.7	49	44.5	16	88.1	96	1414.97	2.03

[1] Treatments are a combination of Lys level increases/decreases (in %) and feed mill processing treatments. Standard diets were conditioned at 76.67°C for 30 seconds and Hygienic diets were conditioned at 87.78°C for 60 seconds with a six-minute retention time in the hygieniser. The hygieniser was used to maintain temperature generated during steam conditioning

[2]A 100% motor load was based on FLA (full load amps) which was 47 amps as seen on the pellet mill motor name plate

[3]Masoneilon valve opening as per the PLC during steady state conditions.

[4]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.

[5]Measurements New Holmen Pellet Tester are where 100 g pelleted samples are subjected to air flow within a perforated chamber for 30 s.

[6]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.

Table 4: Live bird performance and multiple comparison across treatments for d0-7, d0-14, and d0-21

Pelleting Treatment	Formulated Lys Level	d0-7 FI[1] per Bird (kg)	d0-7 FCR[2] (kg/kg)	d0-7 Bird LWG[3] (kg)	d7 Bird Weight (kg)	d0-14 FI per Bird (kg)	d0-14 FCR (kg/kg)	d0-14 Bird LWG (kg)	d14 Bird Weight (kg)	d0-21 FI per Bird (kg)	d0-21 FCR (kg/kg)	d0-21 Bird LWG (kg)	d21 Bird Weight (kg)
	-20	0.116	1.24	0.094	0.132	0.425	1.42	0.301	1.233	1.223	1.77^{a}	0.693^{f}	0.732^{e}
	-10	0.114	1.09	0.103	0.142	0.446	1.28	0.354	0.393	1.310	1.57^{c}	0.834^{cd}	0.873^{bc}
Standard	Ross Level	0.117	1.07	0.110	0.149	0.458	1.21	0.380	0.419	1.328	1.48^{def}	0.900^{ab}	0.938^{a}
	10	0.105	1.04	0.099	0.140	0.443	1.24	0.367	0.408	1.328	1.52^{cde}	0.870^{abc}	0.911^{ab}
	20	0.108	1.01	0.107	0.146	0.434	1.15	0.380	0.419	1.272	1.45^{fe}	0.863^{abc}	0.902^{ab}
	-20	0.117	1.27	0.092	0.131	0.432	1.38	0.321	0.361	1.233	1.68^{b}	0.738^{ef}	0.778^{de}
	-10	0.101	1.15	0.086	0.127	0.426	1.33	0.325	0.366	1.329	1.69^{b}	0.784^{de}	0.825^{cd}
Hygienic	Ross Level	0.112	1.07	0.104	0.143	0.441	1.20	0.369	0.408	1.285	1.53^{cd}	0.841^{bcd}	0.879^{bc}
	10	0.118	1.00	0.118	0.156	0.455	1.18	0.389	0.427	1.305	1.45^{f}	0.902^{a}	0.941^{a}
	20	0.104	1.01	0.103	0.142	0.426	1.14	0.376	0.414	1.255	1.48^{def}	0.851^{abc}	0.889^{ab}
	Treatment *P*-value	0.584	<0.0001	0.122	0.104	0.691	<.0001	<.0001	<.0001	0.007	**<.0001**	**<.0001**	**<.0001**
	Fisher's LSD[4]	0.019	0.066	0.021	0.019	0.040	0.085	0.039	0.037	0.066	0.073	0.060	0.059
	Treatment SEM[5]	0.007	0.024	0.007	0.007	0.014	0.030	0.014	0.013	0.024	0.026	0.022	0.021
Marginal Means													
	Standard Pelleting	0.112	1.10	0.103	0.142	0.441	1.26	0.356	0.396	1.293	1.56	0.832	0.871
	Hygienic Pelleting	0.110	1.09	0.101	0.134	0.436	1.25	0.356	0.395	1.281	1.56	0.823	0.863
	SEM	0.003	0.011	0.003	0.003	0.006	0.013	0.006	0.006	0.017	0.012	0.015	0.015
	-20	0.116	1.25^{a}	0.093	0.132	0.429	1.40^{a}	0.311^{c}	0.350^{c}	1.228^{c}	1.72	0.716	0.755
	-10	0.107	1.12^{b}	0.095	0.135	0.436	1.31^{b}	0.340^{b}	0.380^{b}	1.319^{a}	1.63	0.809	0.849
	Ross Levels	0.114	1.07^{b}	0.107	0.146	0.450	1.21^{c}	0.375^{a}	0.414^{a}	1.307^{ab}	1.50	0.870	0.909
	10	0.112	1.02^{c}	0.109	0.148	0.449	1.21^{c}	0.378^{a}	0.418^{a}	1.317^{a}	1.48	0.886	0.926
	20	0.106	1.01^{c}	0.105	0.144	0.430	1.15^{c}	0.378^{a}	0.417^{a}	1.264^{bc}	1.47	0.857	0.896
	SEM	0.005	0.018	0.005	0.005	0.010	0.021	0.010	0.009	0.011	0.018	0.010	0.009
Main Effects and Interactions													
Pelleting Treatment		0.6871	0.4781	0.6713	0.6809	0.5659	0.4686	0.9754	0.9732	0.5894	0.5303	0.5794	0.5831
Formulated Lys Level		0.4679	**<.0001**	0.0973	0.0679	0.3935	**<.0001**	**<.0001**	**<.0001**	**0.0005**	<.0001	<.0001	<.0001
Pelleting Treatment x Formulated Lys Level		0.4068	0.2925	0.1907	0.2141	0.7368	0.3433	0.3063	0.2984	0.669	**0.0001**	**0.0443**	**0.0431**

[1]Feed Intake [2]Feed Conversion Ratio= Feed:Gain was calculated using mortality weight
[3]Live Weight Gain [4]Fischer's Protected Least Significance Difference Test [5]Standard Error of the Mean [a-e] Means within a column not sharing a common superscript differ significantly (P<.09)

Table 5: d0-21 Apparent Ileal Digestibility (%) Values

Pelleting Treatment	Formulated Lys Level	Asp	Thr	Glu	Pro	Gly	Ala	Cys	Val	Met	Ile	Leu	Lys
Standard	-10	81.691[a]	75.821[a]	86.993[a]	81.937[a]	78.569[a]	83.468[a]	68.997[a]	81.391[a]	94.111[a]	82.996[a]	84.455[a]	86.352[a]
	Ross Level	82.911[a]	78.575[a]	87.704[a]	82.960[a]	79.782[a]	84.646[a]	70.046[a]	82.748[a]	94.602[a]	84.237[a]	85.530[a]	87.135[a]
	10	83.663[a]	80.191[a]	87.698[a]	83.442[a]	80.724[a]	83.925[a]	71.702[a]	83.070[a]	94.058[a]	84.918[a]	85.577[a]	87.411[a]
Hygienic	-10	59.676[b]	42.474[b]	70.307[b]	60.156[b]	50.149[b]	60.479[b]	26.162[b]	56.756[b]	84.253[b]	61.124[b]	63.460[b]	68.052[b]
	Ross Level	84.017[a]	79.691[a]	88.313[a]	83.723[a]	81.405[a]	85.149[a]	72.352[a]	83.540[a]	94.629[a]	82.240[a]	86.164[a]	88.183[a]
	10	84.344[a]	80.793[a]	87.814[a]	84.003[a]	80.899[a]	84.687[a]	72.109[a]	83.032[a]	94.110[a]	84.720[a]	85.873[a]	87.193[a]
	Treatment *P*-value	2.225	3.669	1.893	2.726	3.112	2.531	4.939	2.693	1.068	2.412	2.345	2.010
	Fisher's LSD[1]	6.306	10.397	5.364	7.726	8.821	7.175	13.998	7.632	3.028	6.8352	6.647	5.697
	Treatment SEM[2]	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001
Marginal Means													
	Standard Pelleting	82.766	78.196	87.765	82.78	79.692	84.013	70.248	82.403	94.257	84.05	85.188	86.966
	Hygienic Pelleting	76.012	67.653	82.145	75.961	70.698	76.772	56.874	74.443	90.998	77.028	78.499	81.143
	SEM	1.285	2.118	1.093	1.574	1.797	1.462	2.851	1.555	0.617	1.392	1.354	1.16
	-10	70.683	59.148	78.65	71.046	64.359	71.974	47.579	69.074	89.182	72.06	73.957	77.202
	Ross Levels	83.464	79.133	88.009	83.342	80.414	84.897	71.199	83.144	94.615	84.738	85.847	87.659
	10	84.003	80.492	87.756	83.722	80.811	84.306	71.906	83.051	94.084	84.819	85.75	87.302
	SEM	1.573	2.594	1.338	1.928	2.201	1.79	3.492	1.904	0.756	1.705	1.658	1.421
Main Effects and Interactions													
Pelleting Treatment		0.0005	0.0009	0.0011	0.0034	0.008	0.0009	0.0016	0.0006	0.0004	0.0008	0.001	0.0008
Formulated Lys Level		<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001
Pelleting Treatment x Formulated Lys Level		**<0.0001**	**<0.0001**	**<0.0001**	**<0.0001**	**<0.0001**	**<0.0001**	**<0.0001**	**<0.0001**	**<0.0001**	**<0.0001**	**<0.0001**	**<0.0001**

[1]Fischer's Protected Least Significance Difference Test
[2]Standard Error of the Mean

Table 6: d0-21 Digestible Amino Acid Concentration (DAAC) Values

Pelleting Treatment	Formulated Lys Level	Asp	Thr	Glu	Pro	Gly	Ala	Cys	Val	Met	Ile	Leu	Lys
	-10	1.854^{d}	0.546^{c}	3.236^{c}	0.893^{c}	0.684^{c}	0.851^{c}	0.241^{b}	0.830^{c}	0.649^{d}	0.780^{c}	1.461^{c}	1.062^{d}
Standard	Ross Level	2.015^{c}	0.644^{b}	3.491^{b}	0.962^{bc}	0.742^{bc}	0.923^{bc}	0.259^{ab}	0.910^{bc}	0.71^{b}	0.859^{b}	1.591^{b}	1.141^{c}
	10	2.401^{a}	0.794^{a}	4.017^{a}	1.093^{a}	0.872^{a}	1.001^{a}	0.301^{a}	1.047^{a}	0.762^{a}	1.011^{a}	1.797^{a}	1.364^{a}
	-10	1.426^{e}	0.319^{d}	2.735^{d}	0.734^{d}	0.461^{d}	0.641^{d}	0.094^{c}	0.613^{d}	0.556^{e}	0.617^{d}	1.155^{d}	0.878^{e}
Hygienic	Ross Level	2.092^{bc}	0.646^{b}	3.550^{b}	0.971^{bc}	0.762^{bc}	0.920^{bc}	0.275^{ab}	0.919^{b}	0.672^{c}	0.887^{b}	1.611^{b}	1.190^{bc}
	10	2.185^{b}	0.743^{a}	3.653^{b}	1.025^{ab}	0.801^{ab}	0.957^{ab}	0.274^{ab}	0.947^{b}	0.706^{b}	0.907^{b}	1.657^{b}	1.221^{b}
	Treatment *P*-value	0.053	0.028	0.074	0.033	0.029	0.027	0.018	0.029	0.007	0.024	0.043	0.026
	Fisher's LSD[1]	0.151	0.078	0.2092	0.094	0.0813	0.076	0.05	0.083	0.02	0.069	0.121	0.074
	Treatment SEM[2]	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001
Marginal Means													
	Standard Pelleting	2.09	0.661	3.581	0.983	0.766	0.927	0.267	0.929	0.707	0.883	1.616	1.189
	Hygienic Pelleting	1.901	0.569	3.313	0.91	0.675	0.839	0.214	0.826	0.645	0.803	1.475	1.096
	SEM	0.031	0.016	0.043	0.019	0.017	0.016	0.01	0.017	0.004	0.014	0.025	0.015
	-10	1.64	0.432	2.986	0.814	0.572	0.746	0.168	0.722	0.603	0.699	1.308	0.97
	Ross Levels	2.053	0.645	3.52	0.967	0.752	0.921	0.267	0.915	0.691	0.873	1.601	1.166
	10	2.293	0.769	3.834	1.059	0.836	0.982	0.288	0.996	0.734	0.959	1.727	1.292
	SEM	0.038	0.02	0.052	0.024	0.02	0.019	0.013	0.021	0.005	0.017	0.03	0.018
Main Effects and Interactions													
Pelleting Treatment		<0.0000	<0.0000	<0.0000	0.0096	0.0003	0.0002	0.0006	<0.0000	<0.0001	0.0002	0.0002	<0.0000
Formulated Lys Level		<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001
Pelleting Treatment x Formulated Lys Level		**<0.0001**	**<0.0001**	**0.001**	**0.0483**	**0.0004**	**0.0008**	**<0.0001**	**0.0013**	**0.001**	**0.0009**	**0.0016**	**<0.0001**

[1]Fischer's Protected Least Significance Difference Test
[2]Standard Error of the Mean

Figure 1: d0-21 Live weight gain for standard processing treatment Broken-Line Regression Model

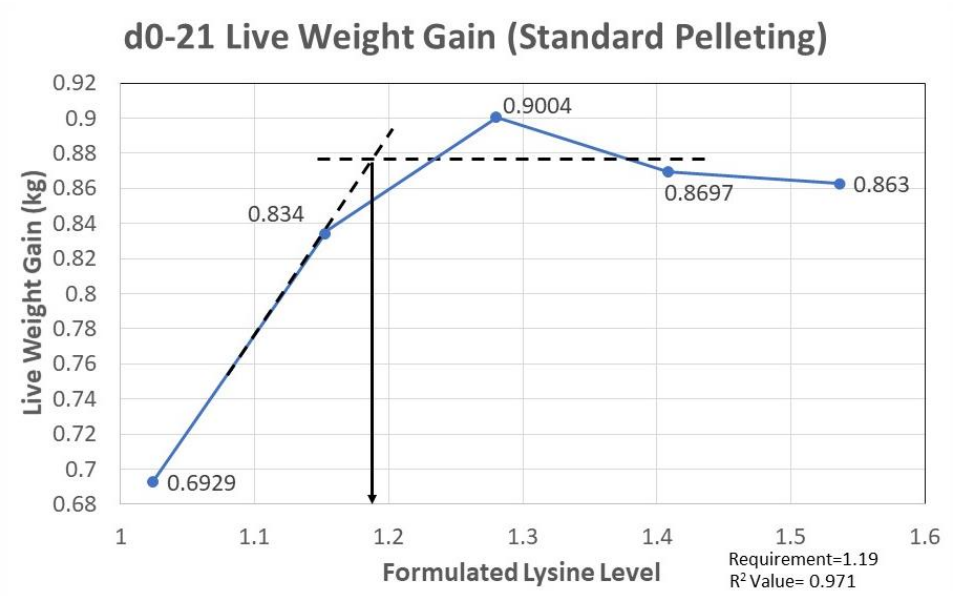


Figure 2: d0-21 Live weight gain for hygienic processing treatment Broken-Line Regression Model

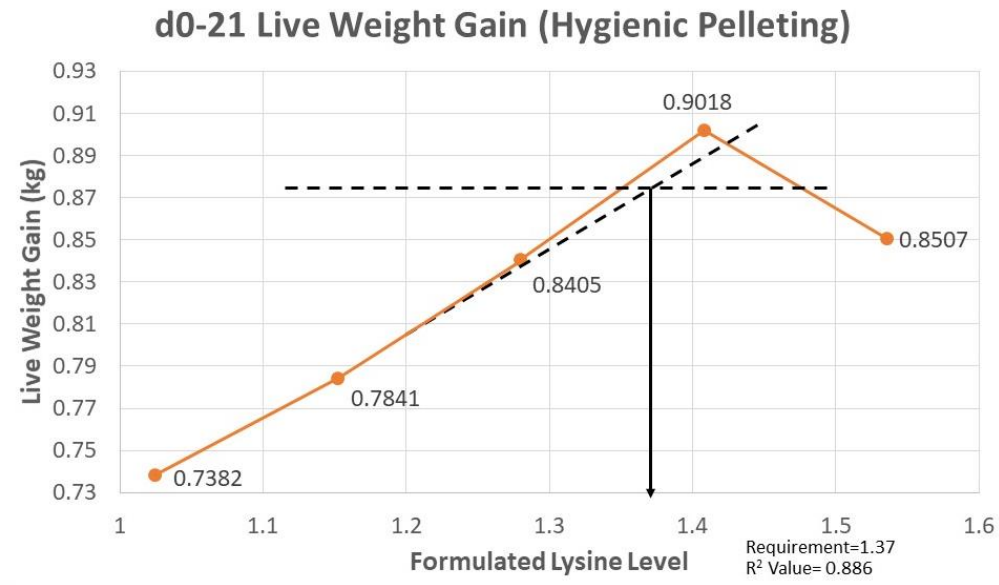
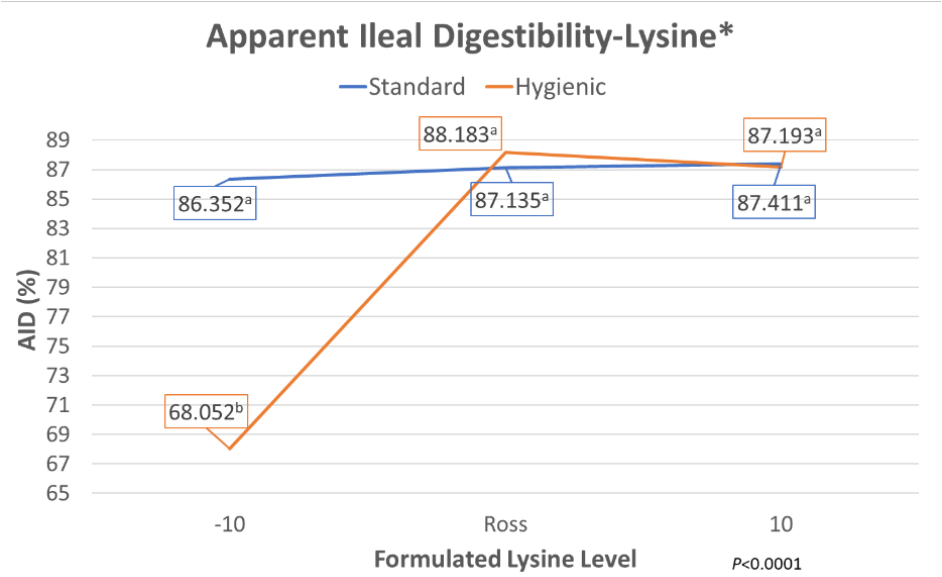
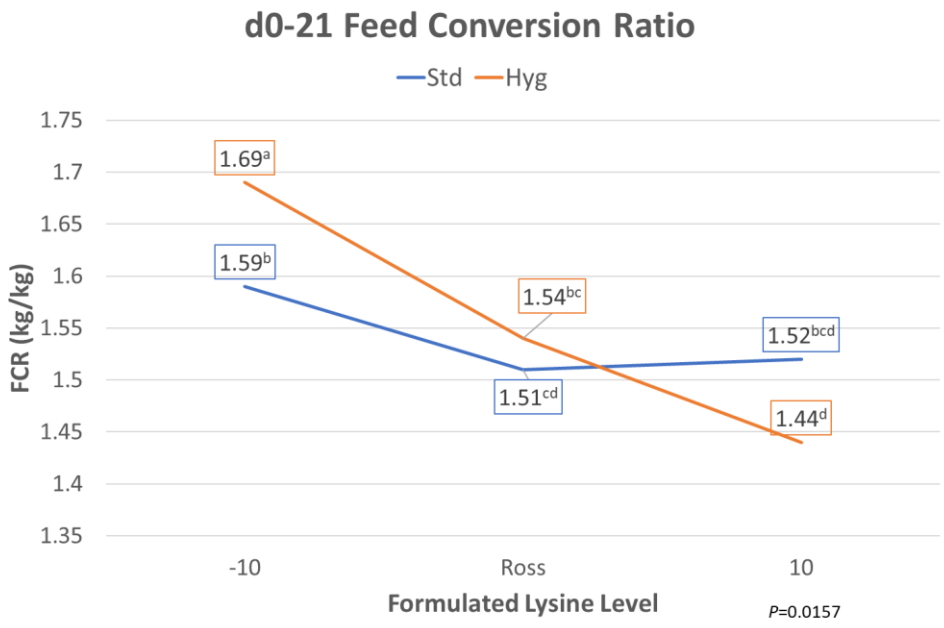


Figure 3: Apparent Ileal Digestibility of Lys, pelleting x formulated lysine level interaction



*All other amino acid AID values displayed a similar interaction including, but not limited to, Cys and Met.

Figure 4: d0-21 Feed Conversion Ratio of the -10, Ross Level, and +10 Standard and Hygienic Pelleting only



CHAPTER THREE

Evaluation of a Novel Phytase on Heat Stability, Broiler Performance, Bone Mineralization, and Mineral Digestibility

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ABSTRACT

The assessment of novel exogenous phytase enzymes that can be added to the mixer prior to pelleting is important for the continual improvement of global broiler production. Two experiments were conducted to determine *in vitro* activity post-pelleting and *in vivo* efficacy post feeding of a novel phytase. The objective of the first study was to determine activity and retention of a novel phytase enzyme (CJ Phytase) and a commercially available phytase (Quantum Blue 5G®) at different 30 second conditioning temperatures (75, 80, 85, and 90˚C) post-pelleting with a 1,000 FTU/kg inclusion rate. The objective of the second study was to determine the effects of these phytase sources conditioned for 30 seconds at the 75˚C conditioning temperature on broiler performance, bone mineralization, and mineral digestibility. Experiment one consisted of a 2 (phytase source) x 4 (conditioning temperature) factorial arrangement. A conditioning temperature main effect (P=0.0003) demonstrated that activity of both phytase products decreased at 85˚C and decreased again at 90˚C. In experiment two, 2,304

male Hubbard x Ross 708 birds were obtained and fed one of eight diets for a 42-day period. Diets included Positive Control, Negative Control deficient in calcium and Available P by 0.2%, and graded levels of NC+ CJ Phytase (250, 500, 1,000, 1,500, and 3,000 FTU/kg). In order to provide a commercially available comparison, an NC + Quantum Blue 5G 500 FTU/kg was also fed. All phytase additions increased AID P, d42 tibia ash percentage, and d0-42 LWG relative to the NC diet (P<0.05). CJ Phytase above 500 FTU/kg and Quantum Blue 500 FTU/kg increased AID Ca relative to the NC diet (P<0.05). The novel CJ phytase demonstrated efficacy in post pellet retention, mineral digestibility, tibia ash percentage, and d0-42 broiler performance.
Key words: Phytase, Enzyme Retention, Feed Efficiency, Broiler Breeder, Mineral Digestibility

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DESCRIPTION OF THE PROBLEM

Challenges presented by phytate phosphorous (**P**), such as decreased broiler performance, decreased bone mineralization, and increased P in excreta that can lead to eutrophication of water ways, can all be alleviated with the supplementation of the exogenous enzyme phytase [3, 5, 7] Phytase is responsible for hydrolyzing phytate P and liberating phosphorous thereby making P more bioavailable in monogastric animals [3].

Phytase inclusion in the diets can be completed by one of two ways: post-pellet application and mixer-added inclusion. The post-pellet application method requires costly equipment and poses concerns about uniform application [4]. To bypass the added expenses and ensure uniformity, mixer-added phytase inclusion is common. However, mixer-added phytase has the potential of becoming denatured during the pelleting process as phytase must be exposed to increased temperatures and moisture [14]. Companies that develop mixer-added phytase

enzymes are interested in developing a heat-stable product that retains activity post-pelleting. In addition to retention, functionality of phytase within the digestive tract must be maintained as phytases are sensitive to pH and can be denatured by proteases [15]. Therefore, *in vivo* testing of the enzyme must be performed [14]. Two studies were conducted. The objective of the first study was to determine activity and retention of a novel, mixer-added phytase enzyme (CJ Phytase) and a commercially available phytase (Quantum Blue 5G®) at different 30 second conditioning temperatures (75, 80, 85, and 90˚C) post-pelleting. The objective of the second study was to determine the effects of these phytase sources conditioned for 30 seconds at the 75˚C conditioning temperature on broiler performance, bone mineralization, and mineral digestibility.

MATERIALS AND METHODS

Experiment one: *In Vitro* Enzyme Activity

Diet Formulation and Batching

All feed was manufactured at the West Virginia University pilot feed mill. A 2x4 factorial was utilized with two enzyme inclusions (A novel enzyme- CJ Phytase and a commercially available enzyme- Quantum Blue 5G®) and four temperatures (75, 80, 85, and 90°C). Phytase was included in a corn/soybean meal (**SBM**) starter poultry diet that was developed using least cost nutritional analysis (**Table 1**). Batching was conducted using a one-ton vertical screw mixer with fat inclusion (soybean oil) at the mixer. A total of 2,721.55 kg feed was batched and separated into six, 453.96kg mash batches.

Enzyme Inclusion

Before final mixing and pelleting, phytase addition was accomplished by adding each phytase to 4.5kg of mash feed from each of the six batches at 1,000FTU/kg. The 4.5kg of feed + phytase mixture was blended in a small paddle mixer for ten minutes. Final mixing was done by mixing the 449.46kg batch with the 4.5kg phytase premix for ten minutes in the one-ton vertical screw mixer.

Feed Manufacture

The experiment was conducted using three replications of 453.96 kg batches of feed that were blocked by time of manufacture. Ten mash samples were obtained during conveyance of feed from the mixer to pellet mill surge bin. Feed was steam conditioned for thirty seconds and extruded through a 4.76 x 38 mm pellet die at approximately 1 metric ton/hr using a 40-horsepower California Pellet Mill (**CPM**). Treatments were first steam conditioned at 75°C. Once steady-state conditions were obtained as indicated by the Programmable-Logic-Control (PLC) Center, hot pellet temperature, ten 500g pellet samples for nutrient analysis, and pellet durability samples were taken. Once all required 75°C samples were collected, temperature was sequentially increased in the conditioner (ie. 80, 85, and 90°C) and the same sample collection process was followed.

Enzyme Activity

Unconditioned mash and cooled pellet samples were sent to a commercial laboratory (Eurofins, Des Moines IA) to determine phytase activity using the AOAC 2000.12 methodology. A 50-g sample was extracted with 500 mL of distilled water containing 0.01% Tween 20 solution. A 100-µL extract was diluted with 300 mL of acetate buffer until a pH of 5.5 was reached. 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 [2].

Experiment Two: Live Bird Performance

Diet Formulation

All feed was manufactured at the West Virginia University pilot feed mill. Corn and SBM-based diets were utilized and were based off the 2019 Ross Nutrient Specifications. A positive control (**PC**) was formulated and negative control (**NC**) diet was formulated to be deficient in calcium (**Ca**) and P by 0.2% relative to the PC (**Table 2**). Eight treatments were manufactured and fed. The PC and NC did not contain exogenous phytase enzyme. The novel CJ phytase was added to the NC diet at concentrations of 250FTU/kg, 500FTU/kg, 1,000FTU/kg, 1,500FTU/kg, and 3,000FTU/kg. Quantum Blue 5G® was added to the NC per the manufacturer's specifications at 500FTU/kg.

Enzyme Inclusion

Before final mixing and pelleting, each phytase enzyme was added to 4.5kg of the NC diet using the required inclusion rates and blended in a small paddle mixer for ten minutes. Final mixing was done by mixing the NC batch required for each treatment at each phase with the 4.5kg phytase premix for ten minutes in the one-ton vertical screw mixer. Fat in the form of soybean oil was then added to the mixer and mixed for ten minutes after enzyme inclusion.

Feed Manufacture

All treatments throughout each grow-out phase were steam-conditioned for 30 seconds at 75°C and extruded through a 4.76 x 38 mm pellet die at approximately 1 metric ton/hr using a

40-horsepower CPM. The starter phase was pelleted, crumbled, and bagged while the grower and finisher phase were pelleted and bagged. Starter diets included titanium dioxide as an indigestible marker. Hot pellet temperature, pellet durability, cooled pellets, and unconditioned mash samples were taken throughout the manufacture process. Feed samples were sent to an outside lab (Midwest Laboratories, Omaha, NE) to be tested for moisture percent, crude protein, total Ca, total P, and titanium dioxide for the starter diets.

Live Bird Performance

A total of 2,304, 1-day old, male Hubbard x Ross 708 (Hubbard 178 LLC, Pikeville, TN; Aviagen, Inc., Huntsville, AL) broiler chicks were obtained from a local commercial hatchery (Longenecker's Hatchery Elizabethtown, PA). On day 1 (d1), birds were weighed and separated into 96 groups of 24 chicks to create uniform initial pen weights. Each group of 24 was placed into one of 96 pens. Treatments were assigned using a randomized complete block design. A block (determined by location within each of three identical floor pen rooms) consisted of eight adjacent pens. A total of twelve blocks were utilized. Standard rearing conditions were used during the entirety of the study. The three grow-out phases consisted of the starter from d1-18, grower from d19-28, and finisher from d29-42. Room temperature for the 1-day-old chicks was set at 32°C and gradually decreased throughout the grow-out period to maximize bird comfort. Feed was placed in feed pans adapted to hoppers and water was supplied through a nipple drinker system; both feed and water were provided for *ad libitum* consumption. Lighting was manipulated through grow-out to ensure that birds had a full GI tract when sampled on d18. From d1 to 3, birds were exposed to 24h light. Light was decreased by one hour from d4-7, four hours from d8-28, and one hour from d29-42 which followed the schedule of a local commercial

poultry integrator. All animals were reared according to protocols established by the West Virginia University Animal Care and Use Committee. (IACUC Protocol #1602000612_R1)

Mortalities were recorded and mortality weights were taken throughout the entirety of the study. Pen weights and residual feed weights were taken on the morning of d18, d28, and d42 for calculations of live weight gain (**LWG**), feed intake (**FI**), mortality adjusted feed conversion ratio (**FCR**), individual bird weight (**IBW**), and mortality percentage. On the morning of d18, five birds per pen were randomly selected for digesta collection. The digestive tract from the Meckel's diverticulum to the ileo-cecal junction was first removed from the bird. De-ionized water was pushed through the lower half of this section to remove digesta. A pooled digesta sample was taken from each pen. Digesta samples underwent lyophilization and were then analyzed for calcium, phosphorus, and titanium dioxide at Midwest Laboratories. On d18 and d42 the left tibia from five birds was excised and used to determine dry-defatted tibia ash %, as well as mg of ash/chick. Excised tibiae were placed in a freezer until tibia ash analysis began. Tibiae were placed in a drying oven at 105˚C for 48 hours. Once dried, tibiae were wrapped in filter paper, placed in a Soxhlet apparatus, and refluxed with petroleum ether for 16 hours. Following fat extraction, the tibiae were allowed to dry. Tibiae were then removed from the filter paper, weighed, and placed in an ashing oven at 600˚C for 18 hours. The inorganic matter remaining was weighed, and ash content was determined [5].

Ca and P Mineral Digestibility Calculation

Mineral digestibility was determined using Apparent Ileal Digestibility (**AID**) calculations [1, 13].

$$AID\ Ca/AID\ P\ coefficient\ (\%) = \left[1 - \left(\frac{Ti_{Diet}}{Ti_{Digesta}}\right) x\ \left(\frac{Nutrient_{Digesta}}{Nutrient_{Diet}}\right)\right] x\ 100$$

Where $Ti_{Digesta}$ and Ti_{Diet} are the analyzed concentrations of Ti (%) in the digesta and diets, respectively, and $Nutrient_{Digesta}$ and $Nutrient_{Diet}$ are the analyzed concentrations of Ca or P in the digesta and diets, respectively [13].

Statistical analysis for Exp 1 and 2

Both experiment one and two utilized randomized complete block designs. Exp 1 utilized a 2 (phytase source) by 4 (conditioning temperature) factorial arrangement with a 453.96kg batch base mix as the experimental unit. In Experiment 2, a pen of 24 chicks was considered the experimental unit and blocking criteria was pen location within the room. Significance was set at $P<0.05$ and tendency at $P<0.10$ Data were analyzed using GLM procedure of SAS [18]. Significant Analysis of Variance (**ANOVA**) results further analyzed differences among means using the Fisher's Least Significant Difference (**LSD**) test and letter superscripts were used to denote differences among treatment means.

RESULTS AND DISCUSSION

Experiment One: In Vitro Enzyme Activity

Feed Manufacture

Feed manufacture data are presented in **Table 3 and 4**. As the temperature increased during conditioning, motor load decreased ($P<0.0001$). This is likely due to increased lubrication at the die due to the increased temperature and moisture level. Fairchild observed a decrease in pellet mill energy consumption when moisture was added to the diet [9, 12]. Increasing temperature also influenced Pellet Durability Index (**PDI**). Pellets that were exposed to an increased conditioning temperature had an increased PDI ($P<0.0001$). Other studies have shown that increasing temperature during steam conditioning will increase PDI [8, 10]. This is also

supported in a study done by Cutlip et. al that showed increasing conditioning temperature increased PDI (P = 0.0001) and modified PDI (P=0.0001) [6].

Enzyme Recovery

Enzyme activity and retention decreased for both the CJ Novel Phytase and Quantum Blue 5G® when steam-conditioned at 85°C and decreased further at 90°C by 22.6% (P=0.0003). Both phytase sources had a mean recovery rate of 90%, 89.1%, 74.7%, and 57.8% at the conditioning temperatures of 75, 80, 85, and 90°C, respectively. Similarly, Homen, et al. [11] reported that as conditioning temperature increased from 82 to 93°C, *in vitro* phytase activity decreased. The CJ Novel Phytase had a tendency to contain higher enzyme retention post-pelleting than the Quantum Blue 5G® (819.17 FTU/kg versus 739.17 FTU/kg, P=0.0905).

Experiment Two: Live Bird Performance

Ca and P Digestibility

All phytase additions increased AID P relative to the NC diet. (**Table 11**) The lowest digestibility coefficient came from the NC, as per design (P<0.05). The highest digestibility coefficient was NC+ Quantum Blue 5G® 500FTU/kg (P<0.05). All treatments produced AID P similar to that of the PC (P<0.05). These enzymes liberated P from phytate enabling an increase in digestibility. Similarly, Rutherford et. al. [17] found that total phosphorus digestibility was significantly (P < 0.05) higher when microbial phytase was added to a rice-bran-based diet. In the current study, the CJ Novel Phytase above 500 FTU/kg and Quantum Blue 5G® 500 FTU/kg increased AID Ca relative to the NC diet, with Quantum Blue 5G® providing the largest AID Ca coefficient. (P<0.05) It can be speculated that a higher inclusion of phytase may be required to liberate Ca from the phytate-Ca complex. A study done by Ravindran et. al. evaluated the influence of supplementation of varying levels of an E. coli-derived phytase on nutrient

utilization in broiler chicks fed corn-based diets containing 3 phytate concentrations (10.4, 11.8, and 13.6 g/kg) and 4 concentrations of phytase (0, 500, 750, and 1,000 FTU/kg of diet). It was reported phytase addition improved Ca digestibility regardless of dietary phytate, but the highest Ca AID coefficient was shown with diets containing 1,000FTU/kg of phytase (P<0.05) [16].

Tibia Mineralization

The overall comparison of treatments for d42 indicated that tibia ash (%) was similar to that of the PC for treatments containing 500 FTU/kg and above (P<0.05). Birds fed the NC had the lowest tibia ash percent and birds receiving the NC+3,000FTU/kg treatment had the highest tibia ash percent (P<0.05). Tibia ash has been shown in previous research to increase with supplementation of microbial phytase (linear, P < 0.01) ranging from 42 to 47% [7].

Live Bird Performance

In each phase of growth and for all the measured performance variables, the PC was significantly different from the NC, except for d0-42 FCR (**Table 9**). For d0-42, the NC had the lowest bird LWG at 2.817kg whereas, the NC+ 500 FTU/kg of CJ Novel Phytase had highest bird LWG, a 0.094kg increase from the PC (P<0.05). Similar to the current study, De Souza et. al. [19] showed an improvement in FI, Weight Gain, and FCR of 4.14, 7.69, and 3.59% respectively, when birds were fed diets supplemented with exogenous phytase as compared to diets that were not supplemented. Viveros and cohorts [20] reported similar body weight gain in birds fed a PC diet and those fed an NC diet with a low P level (0.35% available P) plus the addition of 500 FTU/kg of a phytase enzyme. In the present study, all phytase additions generated d0-42 broiler performance metrics similar to that of the Positive Control Treatment.

Therefore, it can be assumed that the addition of the CJ Novel Phytase can improve broiler performance in diets deficient in Ca and P.

CONCLUSIONS AND APPLICATIONS

1. Enzyme activity and retention decreased for both the CJ Novel Phytase and Quantum Blue 5G® when steam-conditioned at 85°C and decreased further at 90°C.
2. All phytase additions increased AID P, d42 tibia ash percentage, and d0-42 LWG relative to the NC diet.
3. CJ Phytase above 500 FTU/kg and Quantum Blue 500 FTU/kg increased AID Ca relative to the NC diet.
4. All phytase additions generated d0-42 performance metrics similar to that of the Positive Control Treatment.

REFERENCES

1. Adedokun, S.A., P. Jaynes, R.L. Payne, T.J. Applegate, Standardized ileal amino acid digestibility of corn, corn distillers' dried grains with solubles, wheat middlings, and bakery by-products in broilers and laying hens, Poult. Sci, 94 (2015), pp. 2480-2487
2. AOAC International, 2000, AOAC International, Official Methods of Analysis of the AOAC International, (17th ed.), AOAC International, Gaithersburg, MD (2000)
3. Ayres, V.E., M.E. Jackson, S. A Cantley, S.J. Rochell, C.D. Crumpacker, D.T. Lee, B.C. Bodle, W.J. Pacheco, M.S. Rueda, C.A. Bailey, K. Gardner, T.P. Boltz, J.S. Moritz, Multiexperiment evaluation of increasing phytase activity from Optiphos® and Optiphos Plus® on 21-day broiler performance and tibia mineralization, Journal of Applied Poultry Research, Volume 30, Issue 4, 2021, 100210, ISSN 1056-6171
4. Bedford, M.R., Exogenous enzymes in monogastric nutrition—Their current value and future benefits, Anim. Feed Sci. Technol., 86 (1–2) (2000), pp. 1-13
5. Boney, J.W., J.S. Moritz, Phytase dose effects in practically formulated diets that vary in ingredient composition on feed manufacturing and broiler performance, Journal of Applied Poultry Research, Volume 26, Issue 2, 2017, Pages 273-285, ISSN 1056-6171
6. Cutlip, S. E., J. M. Hott, N. P. Buchanan, A. L. Rack, J. D. Latshaw, and J. S. Moritz. 2008. The effect of steam-conditioning practices on pellet quality and growing broiler nutritional value. J Appl Poult Res. 17:249-261.
7. Dilger, R.N., E.M. Onyango, J.S. Sands, O. Adeola, Evaluation of Microbial Phytase in Broiler Diets, Poultry Science, Volume 83, Issue 6, 2004, Pages 962-970, ISSN 0032-5791.

8. Fahrenholz, A. C. 2012. Evaluating factors affecting pellet durability and energy consumption in a pilot feed mill and comparing methods for evaluating pellet durability. PhD Diss. Kansas State University, Kansas.
9. Fairchild, F., D. Greer, Pelleting with precise mixer moisture control, Feed Int., 20 (1999), pp. 32-36
10. Gilpin, A.S., T.J. Herrman, K.C. Behnke, F.J. Fairchild, Feed moisture, retention time, and steam as quality and energy utilization determinants in the pelleting process, App. Eng. in Ag., 18 (2002), pp. 331-338
11. Homan, V.B., J.W. Boney, J.S. Moritz, Effects of steam conditioning temperatures on commercial phytases and subsequent broiler performance and tibia mineralization. Appl. Anim. Sci., 35 (2019), pp. 298-303
12. Hott, J.M., N.P. Buchanan, S.E. Cutlip, J.S. Moritz, The Effect of Moisture Addition with a Mold Inhibitor on Pellet Quality, Feed Manufacture, and Broiler Performance1, Journal of Applied Poultry Research, Volume 17, Issue 2, 2008, Pages 262-271, ISSN 1056-6171
13. Lamp, A.E., A. Mereu, I. Ruiz-Ascacibar, J.S. Moritz, Inorganic feed phosphate type determines mineral digestibility, broiler performance, and bone mineralization, Journal of Applied Poultry Research, Volume 29, Issue 3, 2020, Pages 559-572, ISSN 1056-6171.
14. Loop, S.A., K.G.S. Lilly, L.K. Shires, C.K. Gehring, K.R. Beaman, M.E. Persia, J.S. Moritz, The phytase analytical activity of pelleted diets may not adequately describe efficacy in the bird, Journal of Applied Poultry Research, Volume 21, Issue 3, 2012, Pages 492-501, ISSN 1056-6171

15. Menezes-Blackburn D, Gabler S, Greiner R. Performance of Seven Commercial Phytases in an in Vitro Simulation of Poultry Digestive Tract. J Agric Food Chem. 2015 Jul 15;63(27):6142-9. doi: 10.1021/acs.jafc.5b01996. Epub 2015 Jul 2. PMID: 26111064.
16. Ravindran V., Morel P.C.H., Partridge G.G., Hruby M., Sands J.S. Influence of an E. coli-derived phytase on nutrient utilization in broiler starters fed diets containing varying concentrations of phytic acid. Poultry Sci., 85 (2006), pp. 82-89
17. Rutherfurd, S.M., T.K. Chung & P.J. Moughan (2002) The effect of microbial phytase on ileal phosphorus and amino acid digestibility in the broiler chicken, British Poultry Science, 43:4, 598-606.
18. SAS Institute, 2017, SAS Institute, The SAS System for Windows 2013. Release 9.4, SAS Inst. Inc., Cary, NC (2017)
19. Sousa, JPL de et al. The effect of dietary phytase on broiler performance and digestive, bone, and blood biochemistry characteristics. Revista Brasileira de Ciência Avícola [online]. 2015, v. 17, n. 1 [Accessed 7 March 2022] , pp. 69-76.
20. Viveros, A., A. Brenes, I. Arija, C. Centeno, Effects of microbial phytase supplementation on mineral utilization and serum enzyme activities in broiler chicks fed different levels of phosphorus, Poult. Sci., 81 (2002), pp. 1172-1183.

TABLES

Table 1: Ingredient composition of the diet corn/soybean meal starter poultry diet for Exp. 1 based on 2019 Ross Broiler Starter Nutrient Specifications.

Ingredients	**Inclusion (%)**
Corn	55.99
Soybean meal (46%)	38.53
Soybean oil	1.96
Limestone	1.27
Dicalcium Phosphate	.81
DL – methionine	0.35
Lysine – HCl	0.16
Vitamin/mineral Premix[2]	0.25
Salt	0.35
Sodium bicarbonate	0.10
Threonine	0.12
Choline 70	0.10
Calculated analysis	
ME (kcal/lb)	1375
Crude Protein	22.0
Dig Lysine	1.26
Dig TSAA	0.94
Dig Methionine	0.65
Dig Threonine	0.84
Dig Tryptophan	0.24
Calcium	0.76
Non-phytate Phosphorus	0.28
Sodium	0.18

Table 2: Diet formulation for Starter, Grower, and Finisher phase in Exp. 2. Diets were formulated using Least-Cost Calculations and 2019 Ross Broiler Nutrient Specifications. The starter period lasted from d0-18, the finisher period spanned d19-28, and the finisher period consisted of d29-42.

Ingredients	Starter Diet		Grower Diet		Finisher Diet	
	PC	NC	PC	NC	PC	NC
Corn	55.10	56.88	59.07	60.70	62.33	63.94
Soybean meal (46%)	37.35	37.07	32.63	32.50	29.17	29.04
Soybean Oil	3.68	3.12	4.41	3.87	4.84	4.31
Dicalcium Phosphate	1.64	0.55	1.61	0.52	1.50	0.45
Limestone	0.77	0.91	0.83	0.97	0.79	0.91
L-Methionine	0.34	0.34	0.30	0.30	0.26	0.26
Salt, Plain (NaCl)	0.32	0.32	0.32	0.32	0.32	0.32
Vit/Min Premix	0.25	0.25	0.25	0.25	0.25	0.25
L-Lysine	0.19	0.19	0.21	0.21	0.18	0.18
L-Threonine (80)	0.17	0.17	0.16	0.16	0.13	0.13
Sodium Bicarbonate	0.10	0.10	0.10	0.10	0.10	0.10
Choline 60	0.05	0.05	0.05	0.05	0.05	0.05
L-Valine	0.05	0.05	0.07	0.07	0.06	0.06
Calculated Analysis						
ME (kcal/lb)	1370	1370	1410	1410	1440	1440
Crude Protein	21.66	21.66	20.35	20.40	18.91	18.96
Calcium	0.86	0.66	0.80	0.60	0.75	0.55
Non-Phytate Phosphorus	0.43	0.23	0.40	0.20	0.38	0.18
Sodium	0.17	0.17	0.17	0.17	0.17	0.17
Dig Lysine	1.24	1.24	1.14	1.14	1.04	1.04
Dig Methionine	0.63	0.62	0.57	0.57	0.51	0.51
Dig TSAA	0.93	0.92	0.84	0.84	0.77	0.77
Dig Threonine	0.84	0.84	0.76	0.76	0.70	0.70
Dig Isoleucine	0.89	0.89	0.84	0.84	0.78	0.78

Dig Leucine	1.96	1.97	1.66	1.67	1.57	1.58
Dig Tryptophan	0.24	0.24	0.21	0.21	0.20	0.20
Dig Valine	095	0.95	0.89	0.89	0.82	0.82
Dig Arginine	1.38	1.38	1.33	1.33	1.22	1.23
DEB	265	263	252	252	234	236

Table 3: Descriptive feed manufacture and physical quality data for experiment 1 (Quantum Blue 5G®) with multiple comparison

Phytase Source[1]	Required Temp (°C)	Repetition Number	Ambient Temperature (°C)	Ambient Humidity (%)	Motor Load[2] (%)	Steam Position[3] (Final %)	Hot Pellet Temperature[4] (°C)	NHPT[5]
Quantum Blue 5G®	75	1	30	58	44	12	79	61.9
		2	31.7	49	44	10	76.2	53.0
		3	32.2	47	45	15	76.8	59.5
	80	1	30	58	43	16	84.4	70.3
		2	31.7	49	43	16	80.2	64.9
		3	32.2	47	43	17	80.4	69.2
	85	1	30	58	42	18	85.7	80.6
		2	31.7	49	41	18	84.9	79.1
		3	32.2	47	42	18	85.7	80.1
	90	1	30	58	40	20	90.4	93.7
		2	31.7	49	40	19	87.9	86.9
		3	32.2	47	40	19	89	87.3
Multiple Comparison								
Quantum Blue 5G®	75				44.33^{a}	12.33^{c}	77.33^{d}	58.13^{d}
	80				43.00^{b}	16.33^{b}	81.67^{c}	68.13^{c}
	85				41.67^{c}	18.00^{ab}	85.43^{b}	79.93^{b}
	90				40.00^{d}	19.33^{a}	89.10^{a}	89.30^{a}
P-value					<.0001	0.0010	<.0001	<0.0001
Fisher's LSD[6]					0.7687	2.4908	2.9123	6.2794
Treatment SEM[7]					0.2357	0.7638	0.893	1.9255

[1]The phytase calculated activity is 1,000 FTU/kg. 90.72g of the enzyme was added at the mixer
[2]A 100% motor load was based on FLA (full load amps) that was 47 amps based on the pellet mill motor name plate.
[3]Masoneilon valve opening as per the PLC during steady state conditions.
[4]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.
[5] New Holmen Pellet Tester, measurements are where 100 g pelleted samples are subjected to air flow within a perforated chamber for 30 s.
[6]Fisher's Protected Least Significance Difference Test
[7]SEM= Standard Error of the Mean

*Diets were conditioned at the required temperature for 30 seconds.
*Pellets were manufactured using a 40 horsepower California Pellet Mill and were extruded through a 4.8 x 38mm pellet die.

Table 4: Descriptive feed manufacture and physical quality data for experiment 1 (CJ Novel Phytase) with multiple comparison

Phytase Source[1]	Required Temp (°C)	Repetition Number	Ambient Temperature (°C)	Ambient Humidity (%)	Motor Load[2] (%)	Steam Position[3] (Final %)	Hot Pellet Temperature[4] (°C)	NHPT[5]
CJ Phytase	75	1	32.2	47	45	10	75.6	57.6
		2	32.8	43	44	10	76.7	57.4
		3	31.7	47	44	14	75.9	54.6
	80	1	32.2	47	43	16	81.6	63.6
		2	32.8	43	43	16	80.8	64.7
		3	31.7	47	43	15	80.2	62.6
	85	1	32.2	47	41	17.5	85.1	80.3
		2	32.8	43	42	17	85.3	77.1
		3	31.7	47	41	17	85.1	77.1
	90	1	32.2	47	40	18.5	89.2	85.4
		2	32.8	43	40	18.5	90.3	84.5
		3	31.7	47	40	18	88.7	83.0
Multiple Comparison								
CJ Phytase	75				44.33^{a}	11.33^{c}	76.07^{d}	56.53^{d}
	80				43^{b}	15.67^{b}	80.87^{c}	63.63^{c}
	85				41.33^{c}	17.17^{ab}	85.17^{b}	78.167^{b}
	90				40^{d}	18.33^{a}	89.40^{a}	84.30^{a}
P-value					<.0001	0.0005	<.0001	<.0001
Fisher's LSD[6]					0.7687	2.2738	1.153	2.7927
Treatment SEM[7]					0.2357	0.6972	0.3536	0.8563

[1]The phytase calculated activity is 1,000 FTU/kg. 90.72g of the enzyme was added at the mixer
[2]A 100% motor load was based on FLA (full load amps) that was 47 amps based on the pellet mill motor name plate.

[3]Masoneilon valve opening as per the PLC during steady state conditions.
[4]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.
[5] New Holmen Pellet Tester, measurements are where 100 g pelleted samples are subjected to air flow within a perforated chamber for 30 s
[6]Fisher's Protected Least Significance Difference Test
[7]SEM= Standard Error of the Mean
*Diets were conditioned at the required temperature for 30 seconds.
*Pellets were manufactured using a 40 horsepower California Pellet Mill and were extruded through a 4.8 x 38mm pellet die

Table 5: Descriptive data for Quantum Blue 5G® enzyme recovery following heat treatment in the conditioner using AOAC Method 2000.12 for Exp. 1

Phytase Source[1]	**Temperature (°C)**	**Rep 1**	**Rep 2**	**Rep 3**	**Ave**	**Recovery (%)**
	Mash	1100	1000	1100	1067	
	75	900	890	940	910	85
CJ Phytase	80	890	860	850	867	81
	85	810	870	750	810	76
	90	630	700	740	690	65

[1]The phytase calculated activity is 1,000 FTU/kg. 90.72g of the enzyme was added at the mixer

Table 6: Descriptive data for CJ Phytase enzyme recovery following heat treatment in the conditioner using AOAC Method 2000.12 for Exp.1

Phytase Source[1]	**Required Temperature (°C)**	**Rep 1**	**Rep 2**	**Rep 3**	**Ave**	**Recovery (%)**
	Mash	1000	1100	1000	1033	
	75	840	940	890	890	86
Quantum Blue 5G®	80	880	870	1000	917	89
	85	690	700	660	683	66
	90C	160	530	710	467	45

[1]The phytase calculated activity is 1,000 FTU/kg. 90.72g of the enzyme was added at the mixer

Table 7: Marginal means and main effects/interactions for enzyme activity and retention in experiment 1

Phytase Source (1,000 FTU/kg)	Conditioning Temperature (°C)	Enzyme Retention (FTU/kg)
CJ Novel Phytase	75	910
	80	866.67
	85	810
	90	690
Quantum Blue5G®	75	890
	80	916.67
	85	683.33
	90	466.67
	Treatment *P*-value	0.0012
	Fisher's LSD[3]	188.26
	Treatment SEM[4]	62.8
Marginal Means		
Phytase Source[1]	CJ Novel Phytase	819.17
	Quantum Blue 5G®	739.17
	SEM	31.4
	Fisher's LSD	94.13
Conditioning Temperature (°C)	75	900[a]
	80	891.67[a]
	85	746.67[b]
	90	578.33[c]
	SEM	44.4
	Fisher's LSD	133.12
Main Effects and Interactions		
Phytase Source		0.0905
Conditioning Temperature		**0.0003**
Source x Conditioning Temperature[2]		0.1829

[11]The phytase calculated activity is 1,000 FTU/kg. 90.72g of the enzyme was added at the mixer
[2]Interaction p-value
[3]Standard Error of the Mean [4]Fisher's Protected Least Significance Difference Test

Table 8: Descriptive feed manufacture and physical quality data of the starter, grower, and finisher diets in Exp.2.

Phase	Phytase Source	Treatment (FTU/kg)[1]	Ambient Temperature (°C)	Ambient Humidity (%)	Motor Load) [2] (%)	Steam Position[3] (Final %)	Hot Pellet Temperature[4] (°C)	NHPT [5]	Particle Size[6] (μm)	Standard Deviation for Particle Size	Pellets to Fines Ratio[7] (%)
Starter Phase	CJ Phytase	PC	13.33	68	40	17	76.5	74.6	1441.7	1.97	
		NC	12.78	77	40	18.5	76	67.4	1565.2	1.95	
		NC + 250	13.33	77	40	17.5	76.2	64.9	1536.9	1.83	
		NC + 500	13.33	76	40	16.5	76.5	62.9	1596.1	1.99	
		NC + 1000	13.89	70	40	17	76.4	59.51	1533.3	2.01	
		NC + 1500	14.44	67	40	17	76.7	59.7	1401.5	1.92	
		NC + 3000	13.89	68	40	16.5	76.4	61.4	1329.0	1.92	
	Quantum Blue5G®	NC + 500	13.89	68	40	16.5	76.6	63.2	1400.1	1.86	
Grower Phase	CJ Phytase	PC	23.89	61	42.5	13.5	75.6	58.1			83
		NC	18.89	80	40	16.5	74.9	53.6			77
		NC + 250	21.11	73	40	15.5	74.9	50.8			77
		NC + 500	22.22	69	40	15	75.2	51.7			76
		NC + 1000	22.78	65	40	15	74.8	52			82
		NC + 1500	23.89	62	40	15	75.7	50.3			74
		NC + 3000	23.89	60	40	15	75.8	51.7			79
	Quantum Blue5G®	NC + 500	23.89	62	40	15	76.2	50.2			75
Finisher Phase	CJ Phytase	PC	19.44	47	40	16	75.8	63.0			81
		NC	16.67	50	40	17	75.5	63.8			78
		NC + 250	16.67	49	40	17	75.3	61.0			57
		NC + 500	17.78	45	40	17	75.7	57.1			84
		NC + 1000	18.89	41	40	17	75.7	59.1			74
		NC + 1500	20.56	39	40	17	75.9	59.7			82
		NC + 3000	16.67	59	40	17	75.6	61.1			86
	Quantum Blue5G®	NC + 500	18.33	51	40	17	75.8	57.8			77

[1]An FTU is one phytase unit.
[2]A 100% motor load was based on FLA (full load amps) that was 47 amps based on the pellet mill motor name plate.
[3]Masoneilon valve opening as per the PLC during steady state conditions.
[4]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.
[5]Measurements New Holmen Pellet Tester are where 100 g pelleted samples are subjected to air flow within a perforated chamber for 30 s.

[6]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.
[7]Pellets to fines as a percent of pellets remaining after sifting smaller particles through a #5 screen to total volume of initial feed
*Diets were conditioned at 75°C for 30 seconds.
*Pellets were manufactured using a 40 horsepower California Pellet Mill and were extruded through a 4.8 x 38mm pellet die. Pellets were then crumbled using a roller mill

Table 9: Broiler performance for d0-18 Starter period, d0-28 Grower period, and d0-42 Finisher period in Exp. 2.

Phytase Source	Treatment + concentration (FTU/kg)[1]	d0-18 FI[2] per Bird (kg)	d0-18 FCR[3] (kg/kg)	d0-18 Bird LWG[4] (kg)	d18 Bird Weight (kg)	d0-28 FI per Bird (kg)	d0-28 FCR (kg/kg)	d0-28 Bird LWG (kg)	d28 Bird Weight (kg)	d0-42 FI per Bird (kg)	d0-42 FCR (kg/kg)	d0-42 Bird LWG (kg)	d42 Bird Weight (kg)
	Positive Control	0.862^{ab}	1.19^{bc}	0.721^{ab}	0.768^{ab}	2.460^{a}	1.30^{bc}	1.665^{a}	1.724^{a}	5.523	1.53^{abc}	3.274^{a}	3.397^{a}
	Negative Control	0.813^{c}	1.26^{a}	0.641^{d}	0.689^{d}	2.302^{b}	1.37^{a}	1.478^{c}	1.539^{c}	4.903	1.57^{a}	2.817^{b}	2.939^{b}
	NC + 250	0.838^{bc}	1.20^{b}	0.695^{c}	0.742^{c}	2.409^{a}	1.33^{b}	1.608^{b}	1.668^{b}	5.282	1.54^{ab}	3.154^{a}	3.273^{a}
	NC + 500	0.851^{ab}	1.20^{b}	0.705^{bc}	0.754^{bc}	2.462^{a}	1.31^{bc}	1.660^{ab}	1.723^{a}	5.560	1.51^{bc}	3.368^{a}	3.494^{a}
CJ Novel Phytase	NC + 1,000	0.849^{ab}	1.19^{bc}	0.714^{abc}	0.761^{abc}	2.471^{a}	1.31^{bc}	1.664^{a}	1.725^{a}	5.272	1.54^{ab}	3.134^{a}	3.254^{a}
	NC + 1,500	0.854^{ab}	1.20^{b}	0.712^{abc}	0.756^{abc}	2.471^{a}	1.30^{bc}	1.708^{a}	1.765^{a}	5.619	1.56^{a}	3.317^{a}	3.434^{a}
	NC + 3,000	0.868^{a}	1.18^{bc}	0.729^{a}	0.776^{a}	2.484^{a}	1.30^{bc}	1.703^{a}	1.763^{a}	5.408	1.53^{abc}	3.245^{a}	3.365^{a}
Quantum Blue 5G®	NC + 500	0.842^{ab}	1.16^{c}	0.721^{ab}	0.768^{ab}	2.450^{a}	1.29^{c}	1.683^{a}	1.747^{a}	5.353	1.49^{c}	3.280^{a}	3.402^{a}
	Treatment *P*-value	**0.0046**	**<.0001**	**<.0001**	**<.0001**	**0.0186**	**0.0019**	**<.0001**	**<.0001**	0.1371	**0.027**	**0.0174**	**0.0171**
	Fisher's LSD[5]	0.0265	0.0312	0.0211	0.0218	0.1042	0.0399	0.0543	0.0543	0.4982	0.0473	0.3	0.3004
	Treatment SEM[6]	0.0094	0.0111	0.0075	0.0077	0.037	0.0142	0.0193	0.0193	0.1769	0.0168	0.1065	0.1067

[1] One phytase unit per kg of diet
[2] Feed Intake
[3] Feed Conversion Ratio, Feed:Gain was calculated using mortality weight
[4] Live Weight Gain
[5] Fischer's Protected Least Significance Difference Test

[6] Standard Error of the Mean
[a-d] Means within a column not sharing a common superscript differ significantly (P<.09)

Ross male performance 0 – 17: FI/bird = 739g, Bird weight = 675g, FCR = 1.095, birds in the current study were weighed in the morning of d18.
Ross male performance 0 – 27: FI/bird = 1.888kg, Bird weight = 1.467kg, FCR = 1.287, birds in the current study were weighed in the morning of d28
Ross male performance 0 – 41: FI/bird= 4.499kg, Bird weight= 2.886kg, FCR= 1.559, birds in the current study were weighed in the morning of d42

Table 10: Analyzed nutrient values- moisture, dry matter, protein, and mineral percent in pelleted starter, grower, and finisher diets in Exp.2.

	Phytase Source	Treatment + concentration (FTU/kg)[1]	Moisture (%)[2]	Dry Matter (%)[3]	Crude Protein (%)[4]	Total Phosphorus (%)[5]	Total Calcium (%)[6]	Total Titanium (%)[7]
Starter Phase		Positive Control	13.69	86.31	22.0	0.69	0.96	0.14
		Negative Control	14.24	85.76	22.6	0.53	0.75	0.12
	CJ Novel Phytase	NC + 250	13.96	86.04	22.4	0.54	0.78	0.11
		NC + 500	14	86	21.3	0.49	0.69	0.12
		NC + 1,000	13.94	86.06	22.1	0.49	0.9	0.14
		NC + 1,500	14.08	85.92	20.7	0.52	0.77	0.11
		NC + 3,000	14.06	85.94	22	0.48	0.71	0.11
	Quantum Blue 5G®	NC + 500	14.05	85.95	22.2	0.52	0.8	0.1
Grower Phase		Positive Control	13.01	86.99	20.8	0.68	0.85	
		Negative Control	13.68	86.32	20.1	0.48	0.66	
	CJ Novel Phytase	NC + 250	13.49	86.51	21	0.47	0.71	
		NC + 500	13.64	86.36	20.3	0.45	0.62	
		NC + 1,000	13.52	86.48	20.3	0.47	0.66	
		NC + 1,500	13.22	86.78	20.7	0.48	0.57	
		NC + 3,000	13.64	86.36	20.2	0.46	0.69	
	Quantum Blue 5G®	NC + 500	13.58	86.42	20.9	0.46	0.64	
Finisher Phase		Positive Control	13.74	86.26	18.8	0.57	0.78	
		Negative Control	13.93	86.07	19.4	0.47	0.72	
	CJ Novel Phytase	NC + 250	13.97	86.03	18.6	0.44	0.66	
		NC + 500	13.34	86.66	18.7	0.45	0.64	
		NC + 1,000	13.44	86.56	19.1	0.45	0.68	
		NC + 1,500	13.48	86.52	19.2	0.45	0.65	
		NC + 3,000	13.58	86.42	18.9	0.46	0.63	
	Quantum Blue 5G®	NC + 500	13.25	86.75	19.1	0.44	0.66	

[1] One phytase unit per kg of diet
[2]Determined using AOAC Method 930.15
[3]Determined by subtracting 100- moisture percent
[4]Determined using AOAC Method 990.03
[5]Determined using AOAC Method 985.01 (modified)
[6]Determined using AOAC Method 985.01 (modified)
[7]Determined using EPA Method 6010

Table 11: Tibia mineralization content for d18 and d42 broilers in Exp. 2.

Phytase Source	Treatment+ Concentration (FTU/kg)[1]	d18 Tibia Ash (%)	d18 Tibia Ash (mg/chick)	d42 Tibia Ash (%)	d42 Tibia Ash (mg/chick)	AID[2] Ca	AID P
CJ Novel Phytase	Positive Control	47.87^{a}	635.12^{a}	48.53^{a}	3681.05^{a}	52.94^{cd}	60.03^{c}
	Negative Control	45.99^{e}	500.03^{c}	46.44^{c}	2952.70^{d}	50.21^{d}	48.09^{d}
	NC + 250	46.23^{de}	505.11^{c}	47.57^{b}	3298.67^{c}	58.56^{bcd}	57.54^{c}
	NC + 500	46.84^{cd}	550.42^{bc}	48.36^{a}	3566.90^{ab}	58.08^{bcd}	62.03^{bc}
	NC + 1,000	47.55^{b}	593.77^{ab}	48.55^{a}	3475.75^{b}	62.55^{ab}	66.24^{b}
	NC + 1,500	47.80^{ab}	595.93^{ab}	48.47^{a}	3548.88^{ab}	59.82^{bc}	77.82^{a}
	NC + 3,000	48.34^{a}	618.77^{a}	48.72^{a}	3556.75^{ab}	61.85^{b}	79.83^{a}
Quantum Blue 5G	NC + 500	47.33^{bc}	635.13^{a}	48.38^{a}	3575.75^{b}	70.31^{a}	78.59^{a}
	Treatment *P*-value	**<0.0001**	**<0.0001**	**<0.0001**	**<0.0001**	**0.0005**	**<0.0001**
	Fisher's LSD	0.676	51.3	0.53	142.1	8.381	5.2
	Treatment SEM	0.24	18.22	0.19	50.46	2.98	1.85

[1] One phytase unit per kg of diet
[2]Apparent Illeal Digestibility
[a-d]Means within a column not sharing a common superscript differ significantly (P < 0.05)

CHAPTER FOUR

Resume- Elizabeth Lynch

1206 Lovelace Way Martinsburg, WV 25401 ~ 304-261-9908 ~ ealynch@mix.wvu.edu

OBJECTIVE

An Animal and Nutritional Science Masters student seeking assistantships in Ph.D. program, related to the profession; an individual with excellent verbal and interpersonal communication skills; able to present ideas and represent my personal brand; a student with leadership skills and an interest in parasitology research with livestock

EDUCATION

West Virginia University, Morgantown, WV
M.S: Nutritional and Food Science — Graduation Date: May 2022
With a focus in the poultry species — (Expected)

Delaware State University, Dover, DE
B.S: Animal and Poultry Science Major — Graduation Date: May 2020
With a minor in Biology

WORK EXPERIENCE

West Virginia University Graduate Research Assistant, Morgantown, WV
August 2020- Present

- Works closely with Poultry Specialist to facilitate and conduct research
- Assist in care of hens, roosters, and chicks in floor pen and cage room environments
- Gained the skills of tibia collection and ashing, digesta collection, and poultry processing
- Works in the WVU Pilot Feed Mill for feed manufacture
- Gained the skills of pellet sampling, pellet durability index, and particle size determination
- Filmed and produced teaching/instructional videos for classroom use on the topics of feed testing procedures, poultry/egg/carcass judging, and parts identification as well as a video on the WVU pilot feed mill processes.
- Filmed and produced the first WV FFA Virtual Poultry Judging Competition (2020)
- Assisted with the aseptic transfer of bacteria during plating for research purposes
- Assisted with fellow graduate student research

Delaware State University Undergraduate Research Assistant, Dover, DE
April 2019- 2020

- Worked closely with Small Ruminant Specialist to facilitate and conduct research.
- Assisted in animal care of small ruminants
- Basic farm upkeep
- Assisted with class laboratories
- Assisted with graduate and undergraduate research
- Conducted research using natural anthelmintics for parasite control

- Gained *In Vitro* lab experiences

USDA Internship Program, Country of Belize and Dover, DE
June 2019- August 2019

- Water, air, and soil quality research in Belize
- Obtained cultural experiences
- Acquired laboratory and research experience as well as experience caring and maintaining herds of small ruminant animals

Horses with Hearts Organization, Martinsburg, WV
June 2015-2020

- Equine exercise program internship
- Care, maintenance, and training of therapy horses
- Worked with children and adults with physical and mental disabilities, veterans with PTSD, and people suffering from addiction by teaching them to ride which in turn strengthened fine motor skills, physical impairments, and mental well-being.
- Acquired record keeping and organizational skills

AWARDS

- First place poster presentation at Delaware State University Summer Research Symposium, 2019
- Dean's List Fall 2016 and Fall 2019, President's list Spring 2017-Spring 2019 and Spring of 2020
- DSU Presidential Scholarship Award 2016-2020
- Graduation with honors from Delaware State University (Suma Cum Laude-2020)
- Dr. Donald J Horvath Memorial Scholarship Recipient (2021)

LEADERSHIP

Organizations

- APHA Member 2006–Present
- Horse Judging Team/Coach 2007-2018
- Livestock Judging Team 2008-2018
- Musselman FFA Chapter 2013-2018
- National Society for Collegiate Scholars 2016-2020
- Miss West Virginia America Organization (MWVAO) 2017-Present
- Delaware State University Women's' D1 Equestrian Team Captain 2018-2019
- Student Athletic Advisory Committee. 2018-2020

Special Skills

- Basic MEGA-X program experience
- Research design
- Data collection and analysis
- Scientific report writing
- Leadership and management
- Critical thinking
- Organizational skills
- Public Speaking (presentations for DSU, WVU, MWVAO, FFA and 4-H Events)

CURRICULUM VITAE

Elizabeth A. Lynch

Home Address:	E-mail:	School Address:
1206 Lovelace Way Martinsburg, WV 25401	ealynch@mix.wvu.edu	West Virginia University Morgantown, WV 26505

An Animal and Nutritional Science graduate student seeking assistantships in a Ph.D. program, related to the profession; an individual with excellent verbal and interpersonal communication skills; able to present ideas and represent a personal brand; a student with leadership skills and an interest in animal science parasitology research on novel anthelmintics and their mode of action

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EDUCATION:

B.S. 05/2020	GPA: 3.93	Animal and Poultry Science Major & Biology Minor	Delaware State University (DSU)
M.S 05/2022 (Expected)	GPA: 3.85 (Current)	Food and Nutritional Science Major with a focus on poultry	West Virginia University (WVU)

AWARDS AND SCHOLARSHIPS:

- First place microbiology poster presentation at Delaware State University's Summer Research Symposium, 2019
- Undergraduate Research Assistant in Animal Science Department, 2019
- USDA NIFA Program Research Intern, 2019
- Dean's List Fall 2016 and Fall 2019
- President's list Spring 2017-Spring 2019 and Spring 2020
- DSU Presidential Scholarship Award 2016-2020
- Graduation with honors from Delaware State University (2020)
- WVU Dr. Donald J Horvath Memorial Scholarship Recipient (2021)

CLUBS AND ACTIVITES:

- DSU NCAA D1 Women's Equestrian Team, 2016- 2020
- Team Captain of D1 Sports Team, 2019-2020
- Chi Alpha Sigma Athlete Honor Society, 2018-2020
- National Society of Collegiate Scholars, 2017-2020
- Student Athletic Advisory Committee, 2018-2020
- Miss West Virginia Organization, 2018-Present
- Student member of the American Association of Veterinary Parasitologists, 2020-Present
- Student member of the Poultry Science Association, 2020-Present

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WORK EXPERIENCE:

USDA NIFA Program Research Intern **Summer 2019**

Advisors: Dr. Gulnihal Ozbay and Dr. Kwame K. Matthews

Research Title: The *In Vitro* Anthelmintic Effects of Pumpkin Flesh Extract on *Haemonchus Contortus*

Specific Research Procedures Performed-

- Use of proper farm management and record keeping techniques
- Fecal sample collections
- Trained in the Modified McMaster Fecal Egg Counting Technique
- Larval isolation (Baermann Apparatus Technique) and identification
- Trained to make Phosphate Buffer Saline solution
- Use of proper laboratory etiquette and safety techniques
- Development of *Cucurbita* extracts
- Parasite counting and data collection

Undergraduate Research Assistant Delaware State University **August 2019- June 2020**

Advisor: Dr. Kwame K. Matthews

Research Title: The *In Vitro* Anthelmintic Effects of Pumpkin Flesh Extract on *Haemonchus Contortus* (a continuation)

- Utilized the above skills as well as:
- Worked closely with Small Ruminant Specialist to facilitate and conduct research.
- Assisted in animal care of small ruminants
- Basic farm upkeep
- Assisted with class laboratories
- Assisted with graduate and undergraduate research
- Conducted research using natural anthelmintics for parasite control

Graduate Research Assistant West Virginia University **August 2020-Present**

Advisor: Dr. Joseph Moritz

Research Title: The Effects of Hygienic Pelleting in Diets that Differ in Amino Acid Density on Ross 708 Broiler Performance, Amino Acid Digestibility, and Requirement/ Evaluation of a Novel Phytase on Heat Stability, Broiler Performances, Bone Mineralization, and Mineral Digestibility

- Works closely with poultry nutrition specialist to facilitate and conduct research
- Assists in care of hens, roosters, and chicks in floor pen and cage room environments
- Filmed and produced teaching/instructional videos for classroom use on the topic of WVU pilot feed mill processes.
- Filmed and produced the first WV FFA Virtual Poultry Judging Competition (2020)
- Assisted with the West Virginia Poultry Association Youth Day and WV FFA CDEs

Specific research procedures performed:

- Feed manufacture
- California Pellet Mill operation
- Pellet Durability Index
- Particle size determination
- Pellet Quality Analysis
- Digesta collection and sublimation techniques
- Sublimation chamber operation
- Tibia collection and mineralization techniques
- Poultry processing
- Aseptic bacterial plating techniques

TEACHING EXPERIENCE:

- Teaching Assistant for Poultry Evaluation Course
    - Filmed and produced teaching/instructional videos for classroom use on the topics of feed testing procedures, poultry/egg/carcass judging, and parts identification
- Teaching Assistant for Companion Animal Course
    - Guest lectured on Daily Energy Requirement calculations
    - Tutored course material
- Teaching Assistant for Poultry Production Course
    - Guest lectured on Poultry Disease, Fungal Infections, Bacterial Infections, Parasitic Infections, and Viral Infections
- Teaching Assistant for Advanced Applied Non-Ruminant Nutrition
    - Assisted with hands-on nutrient lab assays, diet formulation, broiler rearing, and experimental design experience

RESEARCH:

Abstracts

-***Elizabeth Lynch***1*, Tyler Niblett1 Jasmine Harris1, Francesss Blake1, Alberta Aryee2, Kwame Matthews1. *In Vitro* Anthelmintic Effects of Pumpkin Flesh Extract on *Haemonchus contortus*, Delaware State University 2019 Summer Research Symposium

-***Elizabeth Lynch*** and Kwame Matthews. The Proposal of *In Vitro* Anthelmintic Effects of Pumpkin Flesh on *Haemonchus contortus*. 2019 Delaware State University Honor's Day

-Kristina Bowen, ***E. Lynch***, V. Ayres, T. Boltz, and J. Moritz. The effect of a dacitic tuff breccia (Azomite®) in corn, soybean, and DDGS based diets that vary in inorganic phosphate source on pellet mill energy consumption, live bird performance and amino acid digestibility. Poultry Science Association, 2021.

-Kristina Bowen, ***E. Lynch***, V. Ayres, T. Boltz, and J. Moritz. Performance and Tibia ash Response of Ross 708 Broilers to increasing concentrations of Optiphos Plus and Quantum Blue Post Pelleting. Poultry Science Association, 2021.

Co- Authored Papers

Bowen, K.M., Jackson, M.E., Ayres, V.E., Boltz, T.P., **Lynch, E.A**., Moritz, J.S. 2022. Performance, Carcass Quality, Tibia ash, and Mineral Digestibility Responses of Ross 708 Broilers to Increasing Dose of Two Commercially Available Mixer-added Phytases. 10.1016/j.japr.2022.100264. Journal of Applied Poultry Research

Presentations

-**"**The Proposal of *In Vitro* Anthelmintic Effects of Pumpkin Flesh Extract on *Haemonchus Contortus*" Delaware State University Honor's Day, April 2019. PowerPoint Presentation

-"The *In Vitro* Anthelmintic Effects of Pumpkin Flesh Extract on *Haemonchus Contortus*" Delaware State University Summer Research Symposium (USDA NIFA Program), July 2019. Poster Presentation

"The Effects of Hygienic Pelleting in Diets that Differ in Amino Acid Density on Ross 708 Broiler Performance and Amino Acid Digestibility" West Virginia University, Davis College of Agriculture Visiting Committee, April 2021. PowerPoint Presentation.

"The Effects of Hygienic Pelleting in Diets that Differ in Amino Acid Density on Ross 708 Broiler Performance and Amino Acid Digestibility" Inaugural Anitox Feed Milling Workshop, September 8, 2021. PowerPoint Presentation

REFERENCES:

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