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Evaluating the Effect of Feed Mixing and Sampling Methodologies on Nutrient Analysis and the Inclusion of a Novel Protease in Broiler Diets

Danielle A. Reese

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Evaluating the Effect of Feed Mixing and Sampling Methodologies on Nutrient Analysis and the
Inclusion of a Novel Protease in Broiler Diets.

Danielle A. Reese

Thesis submitted
to the Davis College of Agriculture, Natural Resources, and Design
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in partial fulfillment of the requirements for the degree of

Master of Science
In
Nutritional and Food Science

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ABSTRACT

Evaluating the Effect of Feed Mixing and Sampling Variables on Nutrient Analysis and the Inclusion of an Novel Protease

Danielle A. Reese

Proper poultry feeding requires a homogenous mixture of macro and micro ingredients to ensure feed nutrients are being supplied to meet bird requirements. Samples of complete feed may be taken during manufacture and analyzed for nutrient content in order to estimate if nutrients are present in feed in accordance to diet formulation. However, variables associated with mixing and feed sampling may influence the results of nutrient analysis, thus over or under estimating the formula comparison. The objective of this study was to vary mix time, sample location, sample number, and sample blending technique on nutrient analysis. Ingredients were mixed in a single screw vertical mixer for either 30 seconds or 10 minutes. Samples were taken from either the pellet die or from the finished feed post cooling, conveyance, and bagging. Samples were blended together from two or 10 samples during manufacture. Blending occurred from either combining samples and mixing by hand or using a sample splitting device. Proximate analysis and free supplemental DL-methionine and L-lysine-HCl were determined on each blended sample and standardized for moisture content. A preliminary study was performed to determine mixer coefficient of variation and showed that ingredients were homogenous when a corn-salt mix was utilized and mixed for 10 minutes ($P < 0.05$). In the primary study, crude fat and free supplemental DL-methionine were affected by mix time ($P < 0.05$). The 10 minute mix time produced values for crude fat and free supplemental DL-methionine that were lower and higher, respectively, compared to the 30 sec mix time and agreed more with the calculated nutrient profile and diet formulation. Free supplemental DL-methionine was also affected by sampling location ($P < 0.05$). Samples obtained at the pellet die were higher and agreed more with the calculated nutrient profile compared to finished feed samples obtained at the sack off bin. Total ash was affected by a sampling location and sampling technique interaction ($P < 0.05$). Samples collected at the pellet die were in closer agreement regardless of sample number; whereas, finished feed samples obtained at the sack off bin were affected more by sample number. These data suggest that mixing and sampling variables can effect nutrient analysis and sampling protocols should be utilized to best estimate if nutrients present in feed are in agreement with diet formulation. The particular conditions of the study suggest that analysis of free supplemental DL-methionine from samples obtained at the pellet die may be the best metric for determining pelleted diet homogeneity and accordance relative to diet formulation.

Exogenous enzymes added at the mixer should demonstrate activity post-pelleting and improve bird performance to justify use in the broiler industry. Past research has shown that diet substrate and the pelleting process can effect enzyme efficacy. The objective of the study was to evaluate the feeding value of a novel protease in unprocessed mash and pelleted diets that were composed of all vegetable protein or an inclusion of animal protein. Treatments were arranged in a $2 \times 2 \times 2$ factorial within a randomized complete block design. Protease inclusion was either 0 or 2%. Diet composition was either all-vegetable protein or an 8% porcine meat and bone meal inclusion. Diets were fed as unprocessed mash or ground pellets that were steam conditioned at 70°C and extruded through a 4×38 mm pellet die. Pellets were ground prior to feeding to

eliminate potentially confounding feed form effects. All diets were formulated to be 85% of crude protein and digestible amino acid recommendations for young broilers. In addition, two unprocessed mash diets representing the all-vegetable protein or 8% porcine meat and bone meal inclusion were formulated to be 100% of crude protein and digestible amino acid recommendations and were fed as controls. Dietary treatments were fed to nine replicate raised wire cages of eight straight-run Hubbard x Cobb broiler chicks for 21 d. Protease activity was confirmed post pelleting and expressed in tyrosine releasing units that compare the absorbance of tyrosine liberated by protease digested casein to a standard curve generated by a tyrosine dilution. Protease and Degree of Processing interacted to effect chick weight gain and feed conversion ratio (FCR) ($P < 0.05$). Protease applied to pelleted diets improved 21 d weight gain by 57g and decreased 21 d FCR by 0.07. Similar improvements were not observed in unprocessed mash diets. Linear contrasts clarified that protease applied to pelleted diets was most beneficial for all vegetable protein compositions ($P < 0.05$). Diet Composition and Degree of Processing interacted to effect FCR ($P < 0.05$). Meat and bone meal inclusion increased feed conversion ratio in unprocessed mash diets but not pelleted diets. Protease efficacy was influenced by diet composition and degree of processing.

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KEY

Chapter 1

1. Amino acids- AA
2. Exogenous enzyme cocktail- EEC
3. Feed conversion ratio- FCR
4. Pellet durability index- PDI
5. Mixer added fat- MAF

Chapter 2

1. Coefficient of variation- CV
2. Dried distillers grains with solubles- DDGS
3. Association of analytical communities- AOAC
4. Crude protein- CP

Chapter 3

1. Tyrosine releasing units per gram- TU/g
2. Mixer added fat- MAF
3. Absorbance- Abs
4. Meat and bone meal- M&B
5. Hot pellet temperature- HPT
6. Exogenous enzyme complex- EEC
7. Feed conversion ratio- FCR

CHAPTER 1: LITERATURE REVIEW

I. Sampling

A. Mixer Uniformity

Feed and feed manufacture account for 60-70% of total production costs associated with a modern poultry operation. Two factors in the feed mill that are critical for optimal broiler performance are utilizing a homogenized, pelleted diet, and ensuring that the feed mill is producing the diet formulated by the nutritionist. Quality control measures may be employed by integrators by taking samples during the feed manufacture process to ensure proper batching and pelleting. One method for ensuring that the diet is homogenized is taking samples from the mixer to test for uniformity [1]. When testing for mixer uniformity, various tracers such as chloride ion, sodium ion, colored iron particles, chromium, and crystalline amino acids can be analyzed for coefficient of variation [1-3]. It is imperative that animals, especially animals with a low feed intake, consume a homogenous diet. This will benefit both animal health and performance [1, 2].

B. Sampling Location and Methodology

Nutritionists formulate diets based on the animal's dietary needs, guaranteed analysis of ingredients, and least-cost formulation. Diets are formulated for specific requirements that coincide with a specific growth period. Samples can be taken during the pelleting process to confirm that the feed mill is producing the correct diet; however, there are many different locations that samples can be taken from and various methodologies that can be utilized. The most accessible locations are post extrusion from the pellet die, the cooler deck, and the finished feed during loading or bagging. After review of literature, no peer-reviewed journal articles were found that compared samples of pelleted feed from multiple locations.

Past literature shows that the number of samples collected may determine the perception of whether or not the mixture is homogenous [1, 2]. For example, collecting two samples and testing for chloride ion content may demonstrate a precise analysis (similar values), but the values may not be accurate (representative of the feed as a whole). Creger demonstrated through a mixing and sampling study that collecting more samples (up to nine) accurately determined diet homogeneity [1]. While analyzing nutrient content of broiler litter, Tasistro et al. states that when data sets consist of relatively small samples, then the sample variance might not provide a good approximation of the population variance [4]. Tasistro et al. also concluded that after the litter had been removed from the house, the samples were less variable because they had undergone a degree of mixing [4].

C. Nutrient Analysis

The Association of Analytical Communities (AOAC) is responsible for developing official methods of analysis (OMA) for microbiological and chemical standards, including feeds, dietary supplements, soil, pharmaceuticals, etc. The most common OMA when dealing with livestock feeds are those involved in proximate analysis: moisture, ash, crude protein, crude fat, and crude fiber. A “crude” value is simply an approximated value. Crude protein is the measure of all protein and non-protein nitrogen. Crude fat is a measure of triglycerides and other lipid-like substances such as waxes, steroids, pigments, etc. Crude fiber is a measure of mainly structural plant material within vegetable components of the diet.

When analyzing for moisture, approximately two grams of feed is placed into a drying oven at 100 degrees Celsius for 16 hours. Once the sample is cooled, it is weighed and the new weight is divided by the original weight, then multiplied by 100 to acquire percent moisture [5]. Ash analysis is performed in a similar manner. Approximately two grams of feed will be placed

in a crucible in a temperature controlled furnace at 600 degrees Celsius for two hours. The sample is weighed back and the new weight is subtracted from the original weight. This value is then divided by the original weight and multiplied by 100 to acquire percent ash [6].

Crude protein, crude fat, and crude fiber analyses can be performed in multiple ways. The most common and well-known method for crude protein is the Copper Catalyst Kjeldahl Method [7]. There are three main steps in this method: digestion, distillation, and titration. Digestion occurs when sulfuric acid and a copper catalyst are added to a one gram sample of feed in a 500-800 mL Kjeldahl flask. The flask is then placed on a burner that is adjusted to bring 250 mL H₂O at 25 degrees Celsius to a boil in five minutes. When white fumes appear in the tube, it is heated for an additional 90 minutes. The flask is allowed approximately 10 minutes to cool, diluted 250 mL with H₂O, and allowed to continue to cool to room temperature. An electronic Kjeldahl apparatus can then be used to automatically titrate the acidic sample solution with a sodium hydroxide solution. The reading from the apparatus can then be used to calculate crude protein, or percent nitrogen [7].

The proper crude fat analysis, or ether extract, depends on the standard. For animal feed, anhydrous ether is prepared as outlined in AOAC Official Method 920.39 [8]. A two gram sample of feed is obtained and placed in a soxhlet apparatus. Ether is allowed to pass through the apparatus at a condensation rate of 2/3 drops per second for 16 hours. The apparatus is removed from the heat source and samples are dried for 30 minutes. Samples are weighed back and the new weight is subtracted from the original weight. This value is divided by the new sample weight, and multiplied by 100 to obtain the crude fat percentage [8].

The most complex proximate analysis procedure is crude fiber. The feed sample will first need to be subject to moisture and ether extract analysis using specific sample weights and

temperatures outlined in AOAC Official Method 692.09 [9]. After ether extraction, the sample is added to an Oklahoma filter screen and the screen is precoated with sulfuric acid and a ceramic fiber mixture. The Oklahoma filter screen is then attached to a vacuum flask with the suction turned on. Boiling H₂O is added and the filter is removed from the beaker to drain excess H₂O from the line. The filter is returned to the beaker and boiling sodium hydroxide is added and boiled for 30 minutes. The beaker is then removed from the heat and the filter screen is precoated a second time. The sample is filtered again using different reagents as described in AOAC Official Method 692.09 [9]. The sample is dried for two hours at 130 degrees Celsius. Once cooled and weighed, the sample is ignited for 30 minutes at 600 degrees Celsius, cooled, and reweighed. Crude fiber percentage is then obtainable using the equations outlined in AOAC Official Method 692.09 [9]. It is important to note that, due to the numerous steps in this method, variability and percent error are very high when the assay is repeated.

D. Methionine and Lysine

Methionine and lysine are the first and second limiting amino acids (AA) for broilers [10]. In commercial diets, these AA are supplemented in crystalline form as DL-methionine and lysine-HCl. However, the price of supplemental AA, along with other ingredients, has been increasing [10]. Higher costs are causing commercial companies to lower their supplemental AA inclusion in poultry diets [10]. This is resulting in impaired FCR and breast meat yield [10-12]. Kalinowski et al. [12] conducted a study that compared methionine and cystine requirements for fast and slow feathering broilers. The authors stated that increasing supplemental methionine concentrations of a diet that was deficient in methionine (70% NRC requirements) improved FCR in a linear manner. Zhai et al. [10] also observed improvements in FCR along with increases in carcass yield and decreased abdominal fat. Similar results have been observed when

experiments were conducted on broilers consuming lysine deficient versus lysine adequate diets [10, 13].

II. Exogenous Enzyme Use in Feed

A. Enzyme Cocktails

Although exogenous enzyme research has been ongoing for decades, the commercial use of exogenous enzymes in feed is much more recent. This is mainly due to the rise in feed ingredient costs and the public's expectations of pollution control from animal agriculture [14, 15]. Generally, the endogenous proteases synthesized in the bird and released in the gastrointestinal tract are considered sufficient for optimal protein and amino acid utilization [14]. Digestibility reports for poultry, however, reveal that endogenous enzymes may not be enough for complete digestion [14-16]. Introduction of exogenous enzymes into the diet aid in utilization of these typically unavailable nutrients, such as large, globular proteins, non-starch polysaccharides, and phytate.

The most established enzyme in both research and commercial poultry markets is phytase [17-19]. Phytase targets molecules of phytate by hydrolyzing the molecule, releasing a usable, inorganic molecule of phosphorus. Other enzyme products, however, are available as exogenous enzyme cocktails (EEC). These EEC contain various combinations of different exogenous enzymes (amylases, proteases, xylanases, glucanase, cellulose, mannanase, and pectinase) [20]. While research shows that unconditioned mash diets containing EEC improve bird performance, different results have been obtained when the diets are pelleted [20]. During pelleting, feed is exposed to high moisture, pressure, and temperatures, which affects both nutrients in the diet and any enzymes used. Some phytases have been shown to lose efficacy at 70°C, xylanases at 105°C,

and glucanases at 75°C [20]. Due to this loss in efficacy, it is thought that live bird performance data may be more useful than enzymatic recovery assays.

B. Heat Stability of Exogenous Enzymes

Pelleting broiler feed has many benefits for both the integrator and the grower, including increases in feed intake and live weight gain, improved FCR, decreased feed selection and wastage by the bird, and improved palatability [22, 23, 26-29]. However, pelleting can also be detrimental due to the exposure to high moisture, pressure, and temperature. Amino acids may become unavailable due to denaturing, loss of nutrients may occur due to attrition throughout the feed mill, and any exogenous enzymes could lose activity due to denaturation [24, 25]. One way to combat performance detriments is to increase the amount of fat added at the mixer to increase lubrication and decrease frictional heat in the pellet die (although this will cause detriments in physical feed properties, such as PDI) [22, 24, 25].

Typically, commercial broiler diets will contain less than 3% mixer-added fat (MAF). Increasing MAF to 3% or more has shown improved broiler performance, improved nutrient utilization, improved enzyme efficacy, decreased post extrusion pellet temperature, and reduced electrical energy usage during pelleting [22, 24]. The decreased post extrusion temperature suggests that fat added at the mixer increases die lubrication, therefore decreasing the frictional heat between the feed and the pellet die [24]. Improved nutrient utilization and enzyme efficacy suggests that decreased frictional heat also decreased the chances of nutrient and enzyme degradation [22]. It has also been proposed that enzyme inactivation is caused by being treated with saturated steam during conditioning [25]. It is possible that decreased pellet quality is caused by MAF coating feed particles and lubricating the pellet die, interfering with starch gelatinization and protein gelation [24, 25].

C. Proteases

Poultry feed often contains complex, indigestible compounds, such as large, globular proteins [26]. Proteases may be added to the diet to increase crude protein and amino acid digestibility. One protease that has received ample attention in research is PWD-1 keratinase, derived from *Bacillus licheniformis* [26-29]. The enzyme was discovered when Shih [26] noticed that shed feathers had completely disintegrated when they were included in the excreta that had undergone laboratory digestion. Since the discovery of PWD-1 keratinase, or Versazyme, it has demonstrated improvements in amino acid digestibility and performance when added to broiler diets [27-29].

Odetallah et al. [27] observed increased body weight and decreased digesta viscosity when diets containing PWD-1 keratinase were fed to broilers. However, authors stated that performance improvements were more prominent when the enzyme was added to control diets (93% NRC requirements for CP) versus experimental diets (78% NRC requirements for CP). Authors speculated that this is because keratinase is a broad-spectrum enzyme that has many different substrates, therefore is able to break down many different peptides, which were more prevalent in the control diet [27]. Wang et al. [28] also observed increased body weight of broilers fed diets incorporating PWD-1 keratinase and formulated to be 95% of NRC requirements for CP. Additionally, the authors stated that broilers fed diets that contained the enzyme demonstrated 1.62% improvement in FCR and 1.68% improvement in breast meat yield [28]. More recently, Stark et al. [29] performed enzymatic recovery assays for PWD-1 keratinase on pelleted diets. Despite low recovery, bird performance still improved when broilers were fed diets containing keratinase. The authors observed increased weight gain and improved FCR (783 to 838 g and 1.73 to 1.56, respectively).

D. Enzymatic Recovery Assays

It is difficult for a commercial company to select an EEC to use in broiler diets due to the high variability in substrate specificity, activity, and stability [25]. Also, *in vitro* results often vary from *in vivo* results because gastrointestinal tract conditions are more dynamic compared to isolated lab conditions [23, 25]. Furthermore, data for the effects of single-enzyme activities are unattainable for EEC [19]. While there has been very little research involving single enzymes, particularly proteases, in poultry diets [18, 19], single protease activity data that is available shows high variation in activity, both *in vitro* and *in vivo*, because there are many different types of proteases that have different substrate specificities. For example, trypsin is an endogenous serine protease that targets peptide bonds involving lysine and arginine [18]. These concerns are causing *in vitro* enzyme assays to be developed that simulate gut conditions; exposing feed with enzyme to different pH and endogenous enzymes present in the gut [25].

Enzyme activity assays have also been developed that are non-specific to a substrate. A non-specific protease assay developed by Sigma-Aldrich, uses casein as a substrate [34]. Tyrosine is liberated when the protease digests the casein [34]. It can be performed using a buffer, casein solution, trichloroacetic acid solution, and a sodium carbonate solution for dilution [31]. The tyrosine absorbance readings from a spectrophotometer indicate how much tyrosine was liberated, and the readings are then used in a calculation to yield protease activity [34].

E. Feed Form

Broiler feed can be produced in three forms: mash, crumble, and pellet. These feed forms have different effects on bird growth and performance [27, 29-32]. Mash is simply a blend of ground ingredients that have not been exposed to conditioning or pelleting. Crumbles have also

been exposed to pelleting and have been ground via roller mill to a particle size between that of a mash and an in-tact pelleted diet.

Since feed and feed manufacture represent 60-70% of the costs of broiler production, it is imperative that integrated poultry growers are able to efficiently produce feed that birds are able to efficiently utilize [26, 33]. Utilizing an improved feed form, such as pellets, has demonstrated increased live weight gain, increase feed intake, improved feed conversion ratio (FCR), improvements in production energy, decreased ingredient segregation, decreased feed wastage, and a decrease in pathogens when compared to a mash diet [30-33]. The degree that these improvements are observed depends on the pellet quality [30-33]. Increasing pellet quality by 10% has been shown to increase carcass weight by 10 g, increase breast weight by 4 g, and improve FCR by 0.0004 for Cobb x Cobb 500 broilers despite an increase in feed intake [32, 33]. Glover et al. [31] also observed performance benefits, including a 3 point FCR improvement and decreased feed intake coupled with similar live weight gain, for Hubbard x Cobb 500 broilers fed an improved pellet quality diet (70% crumble/pellet) verses a standard pellet quality diet (50% crumble/pellet). Birds fed pelleted diets are able to consume the same amount of feed as those fed a mash diet in a shorter amount of time [30]. Reduced eating time decreases energy expenditure and birds are able to utilize more energy for growth and maintenance [30]. Since birds fed pelleted diets are able to consume a set amount of feed more quickly, they are able to consume more “meals” per day than birds fed a mash diet [30]. These observations could explain why birds fed pelleted diets consume more feed while demonstrating improved performance, and could justify costs associated with the pelleting process [30].

References

1. Creger, C. R. 1957. A study of distribution of micro-ingredients in mixed feeds. MS Thesis. Kansas State Univ., Manhattan.

2. McCoy, R. A., K. C. Behnke, J. D. Hancock, and R. R. McElhiney. 1994. Effect of mixing uniformity on broiler chick performance. *Poult Sci.* 73:443-451.
3. Clark, P. M., K. C. Behnke, and D. R. Poole. 2007. Effects of marker selection and mix time on the coefficient of variation (mix uniformity) of broiler feed. *J Appl Poult Res.* 16:464-470.
4. Tasistro, A. S., D. E. Kissel, and P. B. Bush. 2004. Sampling broiler litter: how many samples are needed? *J Appl Poult Res.* 13:163-170.
5. Official Methods of Analysis of AOAC INTERNATIONAL (2000) 17th Ed., AOAC INTERNATIONAL, Gaithersburg, MD, USA, Official Method 934.01.
6. Official Methods of Analysis of AOAC INTERNATIONAL (2000) 17th Ed., AOAC INTERNATIONAL, Gaithersburg, MD, USA, Official Method 942.05.
7. Official Methods of Analysis of AOAC INTERNATIONAL (2000) 17th Ed., AOAC INTERNATIONAL, Gaithersburg, MD, USA, Official Method 984.13.
8. Official Methods of Analysis of AOAC INTERNATIONAL (2000) 17th Ed., AOAC INTERNATIONAL, Gaithersburg, MD, USA, Official Method 920.39.
9. Official Methods of Analysis of AOAC INTERNATIONAL (2000) 17th Ed., AOAC INTERNATIONAL, Gaithersburg, MD, USA, Official Method 692.09.
10. Zhai, W., E. D. Peebles, M. W. Schilling, and Y. Mercier. 2016. Effects of dietary lysine and methionine supplementation on Ross 708 male broilers from 21 to 42 d of age (I): growth performance, meat yield, and cost effectiveness. *J Appl Poult Res.* 0:1-15.
11. Hickling, D., W. Guenter, and M. E. Jackson. 1990. The effects of dietary methionine and lysine on broiler chicken performance and breast meat yield. *Can J Anim Sci.* 70:673-678.
12. Kalinowski, A., E. T. Moran, Jr., and C. Wyatt. 2003. Methionine and cystine requirements of slow- and fast-feathering male broilers from zero to three weeks of age. *Poult Sci.* 82:1423-1427.
13. Wang, H., C. Zhang, Y. Mi, and M. T. Kidd. 2014. Copper and lysine amino acid density responses in commercial broilers. *J Appl Poult Res.* 23:470-477.
14. Freitas, D. M., S. L. Vieira, C. R. Angel, A. Favero, and A. Maiorka. 2011. Performance and nutrient utilization of broilers fed diets supplemented with a novel mono-component protease. *J Appl Poult Res.* 20:322-334.

15. Angel, C. R., W. Saylor, S. L. Vieira, and N. Ward. 2011. Effects of a monocomponent protease on performance and protein utilization in 7- to 22-day-old broiler chickens. *Poult Sci.* 90:2281-2286.
16. Wang, X. and C. M. Parsons. 1998. Effect of raw material source, processing systems, and processing temperatures on amino acid digestibility of meat and bone meals. *Poult Sci.* 77:834-841.
17. Yan, F., C. A. Fritts, and P.W. Waldroup. 2003. Evaluation of modified dietary phosphorus levels with and without phytase supplementation on live performance and fecal phosphorus levels in broiler diets. 1. full-term feeding recommendations. *J Appl Poult Res.* 12:174-182.
18. Shelton, J. L. and L. L. Southern. 2006. Effects of phytase addition with or without a trace mineral premix on growth performance, bone response variables, and tissue mineral concentration in commercial broilers. *J Appl Poult Res.* 15:94-102.
19. Loop, S. A., K. G. S. Lilly, L. K. Shires, C. K. Gehring, K. R. Beaman, M. E. Persia, and J. S. Moritz. 2012. The phytase analytical activity of pelleted diets may not adequately describe efficacy in the bird. *J Appl Poult Res.* 21:492-501.
20. Beaman, K. R., K. G. S. Lilly, C. K. Gehring, P. J. Turk, and J. S. Moritz. 2012. Influence of pelleting on the efficacy of an exogenous enzyme cocktail using broiler performance and metabolism. *J Appl Poult Res.* 21:744-756.
21. Malathi, V. and G. Devegowda. 2001. In vitro evaluation on nonstarch polysaccharide digestibility of feed ingredients by enzymes. *Poult Sci.* 80:302-305.
22. Corey, A. M., K. G. S. Wamsley, T. S. Winowiski, and J. S. Moritz. 2014. Effects of calcium lignosulfonate, mixer-added fat, and feed form on feed manufacture and broiler performance. *J. Appl. Poult. Res.* 23:418-428.
23. Chewning, C. G., C. R. Stark, and J. Brake. 2012. Effects of particle size and feed form on broiler performance. *J. Appl. Poult. Res.* 21:830-837.
24. Gehring, C. K., K. G. S. Lilly, L. K. Shires, K. R. Beaman, S. A. Loop, and J. S. Moritz. 2011. Increasing mixer-added fat reduces the electrical energy required for pelleting and improves exogenous enzyme efficacy for broilers. *J. Appl. Poult. Res.* 20:75-89.
25. Lamp, A. E., A. M. Evans, and J. S. Moritz. 2015. The effects of pelleting and glucanase supplementation in hulled barley based diets on feed manufacture, broiler performance, and digesta viscosity. *J. Appl. Poult. Res.* 24:295-303.
26. Shih, J. C. H. 1993. Recent development in poultry waste digestion and feather utilization – a review. *Poult Sci.* 72:617-620.

27. Odetallah, N. H., J. J. Wang, J. D. Garlich, and J. C. H. Shih. 2003. Keratinase in starter diets improves growth of broiler chicks. *Poult Sci.* 82:664-670.
28. Wang, J. J., J. D. Garlich, and J. C. H. Shih. 2006. Beneficial effects of versazyme, a keratinase feed additive, on body weight, feed conversion, and breast yield of broiler chickens. *J Appl Poult Res.* 15:544-550.
29. Stark, C. R., B. E. Spencer, J. C. H. Shih, C. G. Chewning, and J. J. Wang. 2009. Evaluation of keratinase stability in pelleted broiler diets. *J Appl Poult Res.* 18:30-33.
30. Jensen, L. S., L. H. Merrill, C. V. Reddy, and J. McGinnis. 1962. Observations on eating patterns and rate of food passage of birds fed pelleted and unpelleted diets. *Poult. Sci.* 41:1414-1419.
31. Glover, B. G., K. L. Foltz, I. Holásková, and J. S. Moritz. 2016. Effects of modest improvements in pellet quality and experiment pen size on broiler chicken performance. *J. Appl. Poult. Res.* 25:21-28.
32. Lemons, M. E. and J. S. Moritz. 2016. The effect of feeder space access and crumble- or pellet-to-fine ratio on 38-day-old broiler performance. *J. Appl. Poult. Res.* 25:12-20.
33. Lilly, K. G. S., C. K. Gehring, K. R. Beaman, P. J. Turk, M. Sperow, and J. S. Moritz. 2011. Examining the relationships between pellet quality, broiler performance, and bird sex. *J. Appl. Poult. Res.* 20:231-239.
34. Cupp-Enyard, C. 2008. Sigma's non-specific protease activity assay – casein as a substrate. *J. Vis. Exp.* 19:889.

CHAPTER 2: Effect of mixing and sampling method on pelleted feed nutrient analysis and diet formulation accordance

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SUMMARY Proper poultry feeding requires a homogenous mixture of macro and micro ingredients to ensure feed nutrients are being supplied to meet bird requirements. Samples of complete feed may be taken during manufacture and analyzed for nutrient content in order to estimate if nutrients are present in feed in accordance to diet formulation. However, variables associated with mixing and feed sampling may influence the results of nutrient analysis, thus over or under estimating the formula comparison. The objective of this study was to vary mix time, sample location, sample number, and sample blending technique on nutrient analysis. Ingredients were mixed in a single screw vertical mixer for either 30 seconds or 10 minutes. Samples were taken from either the pellet die or from the finished feed post cooling, conveyance, and bagging. Samples were blended together from two or 10 samples during manufacture. Blending occurred from either combining samples and mixing by hand or using a sample splitting device. Proximate analysis and free supplemental DL-methionine and L-lysine-HCl were determined on each blended sample and standardized for moisture content. A preliminary study was performed to determine mixer coefficient of variation and showed that ingredients were homogenous when a corn-salt mix was utilized and mixed for 10 minutes ($P < 0.05$). In the primary study, crude fat and free supplemental DL-methionine were affected by mix time ($P < 0.05$). The 10 minute mix time produced values for crude fat and free supplemental DL-methionine that were lower and higher, respectively, compared to the 30 sec mix time and agreed more with the calculated nutrient profile and diet formulation. Free supplemental DL-methionine was also affected by sampling location ($P < 0.05$). Samples obtained at the pellet die were higher and agreed more with the calculated nutrient profile compared to finished feed samples obtained at the sack off bin. Total ash was affected by a sampling location and sampling technique interaction ($P < 0.05$). Samples collected at the pellet die were in closer agreement regardless of

sample number; whereas, finished feed samples obtained at the sack off bin were affected more by sample number. These data suggest that mixing and sampling variables can effect nutrient analysis and sampling protocols should be utilized to best estimate if nutrients present in feed are in agreement with diet formulation. The particular conditions of the study suggest that analysis of free supplemental DL-methionine from samples obtained at the pellet die may be the best metric for determining pelleted diet homogeneity and accordance relative to diet formulation.

Keywords: mixing time, blending technique, sample number, sample location, proximate analysis

DESCRIPTION OF PROBLEM

Feed and feed manufacture account for 60-70% of the total costs of production associated with commercial poultry rearing. The use of a homogenized, pelleted diet optimizes bird performance and maximizes production efficiency [1-3]. A homogenized diet has been described as ingredients being mixed to achieve nutrient coefficient of variation (CV) of less than 10% [3]. McCoy et al. [2] evaluated mixer effects on 28 d broiler performance and observed increases in average daily gain (23.6 vs. 30.3 g), average daily feed intake (43.1 vs. 52.7 g), gain:feed (0.548 vs. 0.575) and a decrease in mortality (12.0 vs. 0%) as mixer revolutions and presumably diet homogeneity increased, respectively.

Different nutrients and additives have been used as markers to determine mixer uniformity. Accuracy of the laboratory assay for the specific marker, as well as ease and cost of the assay, are important for marker selection [3]. Pfost et al. [4] suggested selecting ingredient markers that have similar physical properties, and not to utilize nutrient markers that are found in multiple ingredients (e.g., crude protein). Clark et al. [3] conducted a mixer uniformity study that utilized a 454 kg capacity double ribbon mixer and complete feed. These authors found that analyses for nutrients found in multiple ingredients (crude protein, chloride ion, phosphorus, and manganese) did not differ with varying mix times. However, analyses for DL-methionine and L-lysine-HCl, demonstrated that mixer CV decreased as mix time increased from 30 seconds to 5 minutes (23.86 to 9.47% and 19.75 to 8.7%, respectively).

In commercial feed manufacture, once diets are mixed to a uniform consistency, feed will be conveyed and packaged as mash or pelleted, cooled, and packaged prior to transport and feeding. Commercial broilers are provided pelleted diets due to ease of handling, decreased feed wastage at the feed pan, decreased energy expenditure in prehension of feed, decreased

ingredient selection, and decreased probability of microbial contamination due to exposure to high temperature and pressure [5]. Conditions of high temperature, pressure, and moisture cause the gelation of proteins and gelatinization of starch that enable pellet formation [5]. However, attrition of pellets can occur during conveyance, cooling, packaging, transportation to the grow-out-barn, and conveyance through the barn. Attrition may lead to significant ingredient and subsequent nutrient segregation of feed. Confirmation that the feed mill is accurately producing an intended diet formulation could be an important control point for feed quality assurance.

Depending on the method of pellet sampling in the feed mill, and the particular nutrient analysis, the comparison to calculated nutrients and ingredient inclusions of the formulation may be over or underestimated. Data does not exist on proper pellet sampling technique within the feed mill to best determine pelleted feed homogeneity and accordance to diet formulation. The objective of this study was to manipulate mixing and pellet sampling variables in the feed mill and compare sample nutrient analyses with diet formulation in order to recommend a sampling method with increased accuracy.

MATERIALS AND METHODS

Preliminary Study

A preliminary study was conducted to determine the proper mix time of a 0.91 MT vertical screw horizontal mixer [6] using a corn-salt diet at the West Virginia University pilot feed mill. Three 136 kg batches of feed containing 3% iodized salt (NaCl) were mixed for 30 sec and three similarly formulated 136 kg batches of feed were mixed for 10 min. Ten samples were taken from 10 different locations in the mixer after each mixing period (30 sec and 10 min) and analyzed for chloride ion concentration using Quantab Test Strips [7]. This procedure was

repeated using similar quantities and replicates of a complete broiler diet (corn and soybean meal based). Coefficient of variation was calculated using the obtained samples and following equation:

$$\%CV = s^2/m \times 100$$

$$m = (\sum x_i)/n$$

$$s^2 = [\sum (x_i^2) - nm^2]/n-1$$

Diet Formulation and Feed Manufacture

Proximate analysis was conducted on base ingredients (corn, soybean meal, meat and bone meal, and DDGS) (Table 1), and incorporated into the feed formulation program ingredient matrix. A corn and soybean meal based diet (Table 2) was formulated based on average nutrient concentrations in the U.S. for broiler chickens in the finisher phase [8]. Corn and soybean meal were ground via hammer mill and augured into the mixer. Additional ingredients were added directly into a top access door of the mixer. All fat in the diet formulation was in the form of soybean oil. Three 136 kg batches of feed were mixed for 30 seconds and three 136 kg batches of feed were mixed for 10 minutes. Each batch, post mixing, was steam conditioned at 80°C for 10 seconds and extruded through a 4 x 38 mm pellet die using a 40-horsepower California Pellet Mill [9]. Pellet production rate was approximately 0.91 MT/hr and pellet conveyance post extrusion was accomplished with a series of flat bottom drag-chain conveyers and a bucket elevator. Pellets were cooled using a horizontal belt cooler and ambient air. During pelleting, samples were taken post extrusion from the pellet die as hot pellets and post conveyance and cooling as finished feed. Two or 10 samples were collected at equally spaced time intervals.

Each sample was approximately 300 g. Hot pellet samples were immediately cooled using ambient air and a fan for 12 minutes. Samples were pooled by placing samples into a general sample bag specific to sample location and time interval. Subsamples were obtained by blending pooled samples by hand or using a Humboldt sample splitting device [10]. Each subsample was approximately 300 g.

Sample Analysis

Proximate analysis [11-14] and free supplemental amino acid analyses for DL-methionine and L-lysine HCl [15, 16] were conducted on all subsamples by commercial laboratories [17, 18]. All nutrient analysis followed proper AOAC methodologies [9-14] and moisture was adjusted to 15% to be similar to calculated moisture of the diet formulation (Table 2).

Statistical Analysis

A 2 x 2 x 2 x 2 factorial arrangement within a completely randomized design was used to explore the main effects and interactions of mix time, sampling location, sampling number, and blending technique. The experimental unit was a 300 g subsample of feed. Treatments were replicated three times. Analyses were completed using the GLM ANOVA procedure from the SAS Institute [19]. Means were considered significantly different at $\alpha \leq 0.05$.

RESULTS AND DISCUSSION

Results of the preliminary study that used a corn-salt mix and chloride ion analysis, suggested that a 10 minute mix time produced a superior mixer CV compared to a 30 sec mix

time ($P < 0.05$, Table 3). Chloride ion analysis CV of complete corn and soybean meal based diets did not differ between mix times ($P > 0.05$), likely due to improper marker selection for the particular diet formulation. These results are in agreement with Clark et al. [3], who found crude protein, chloride ion, and phosphorus were not adequate predictors of mixer CV. Our assumption was that complete corn and soybean meal based diets mixed for 10 minutes produced a homogenous mixture of ingredients.

Calculated nutrient profiles from the feed formulation program determined from analyzed nutrients of ingredients in the formulation and diet inclusion were considered an accurate standard for comparison of subsample analytical nutrient values. In order to provide a concise presentation of results, only marginal means of the main effects and significant interactions were presented in Table 4. Crude fat and free supplemental DL-methionine were affected by mix time ($P < 0.05$). The 10 minute mix time produced crude fat and free supplemental DL-methionine that were lower and higher respectively compared to the 30 sec mix time and agreed more with the calculated nutrient profile and diet formulation (Table 2). In addition, some of the pellets that were mixed for 30 sec had a “fir tree” appearance. This characteristic of pellets has been linked to excessive lubrication at the mash and pellet die interface, and in the current study was likely associated with improper dispersion of soybean oil within the mix [20]. These results are in agreement with Creger [1], who utilized mix times ranging between 30 seconds and 20 minutes. Using the chloride ion analysis, the author concluded that diets were homogenous when mixed for at least five minutes. Another mixer uniformity study performed by McCoy et al. [2] utilized between five and 80 mixer revolutions, and resulted in mixer CV $<10\%$ when at least 35 revolutions were utilized. More recently, Clark et. al [3] suggested that mixer CV significantly

decreased as mix time increased from 30 seconds to 5 minutes and samples were analyzed for free supplemental DL-methionine and L-lysine-HCl [3].

Free supplemental DL-methionine was also affected by sampling location ($P < 0.05$). Samples obtained at the pellet die were higher and agreed more with the diet formulation compared to finished feed samples obtained post conveyance and cooling. Total ash was affected by a sampling location and sampling technique interaction ($P < 0.05$). Samples collected at the pellet die were in closer agreement regardless of sample number; whereas, finished feed samples obtained post conveyance and cooling were affected more by sample number. These results were likely due to pellet attrition and ingredient separation from feed conveyance through the feed mill. Feed auguring or transportation via drag-chain has been shown to segregate feed particles, ingredients, and nutrients [21, 22]. Tang et al. [21] examined segregation patterns in hen houses that contained auger systems and drag-chain systems; the authors observed top-to-bottom and side-to-side segregation within feed troughs. In top-to-bottom segregation, small, fine particles percolated to the bottom of the troughs while large, coarse particles remained at the top [21]. In side-to-side segregation, fine particles remained in the center of the trough while large particles separated to either side of the trough [21]. Moritz [22] observed that feed form segregation also occurs in commercial broiler house feed lines. Pelleted feed was shown to have improved conveyance to end feed pans whereas fines filtered at higher concentrations into feed pans more proximal to the in-house feed hopper.

Crude protein was affected by sampling technique ($P < 0.05$). Subsamples produced from two samples placed in a general sample bag provided a lower crude protein value and agreed more with the calculated nutrient profile than subsamples produced from 10 samples placed in a general sample bag (Tables 4 and 2). Intuitively, the number of samples collected

should improve agreement to a calculated nutrient profile if the mix is not originally homogenous. However, crude protein was not affected by mix time i.e. lack of homogeneity. Therefore, crude protein variation in this study may be more associated with the marker being found in several ingredients and analytical error. Free supplemental L-lysine-HCl was not affected by any main effect or interaction in the current study. This result was in disagreement with Clark et al. [3], who observed a significant decrease in free supplemental L-lysine-HCl CV when mixing time was increased. The main effect, blending technique, did not influence nutrient analysis per se or interact with other main effects ($P > 0.05$, Table 4).

Chloride ion analysis in the preliminary study, and crude fat, free supplemental DL-methionine analysis, and physical appearance of pellets in the factorial study suggest that the 10 min mix time produced a homogenous mix of ingredients. Sampling location influenced nutrient analyses and agreement with diet formulation likely due to pellet attrition and ingredient segregation that occurs during conveyance through the feed mill. Sampling number had a more minor effect that was potentially confounded by marker selection and nutrient analysis and sample blending technique had no effect. We speculate that analysis of free supplemental DL-methionine from a sample obtained post extrusion from the pellet die may estimate diet homogeneity and indicate accordance to diet formulation. This sampling method has three major advantages: 1) free supplemental DL-methionine analysis of samples obtained at the pellet die would not be confounded by segregation due to feed conveyance; 2) free supplemental DL-methionine analysis comparison to diet formulation would be based on formulation inclusion and not nutrient analysis of several ingredients; and 3) free supplemental DL-methionine analysis would require a low number of samples (2 samples provided similar results to 10 samples in this study).

CONCLUSIONS AND APPLICATIONS

1. Within the parameters of this study, pellets sampled at the pellet die and analyzed for free supplemental DL-methionine, standardized for percent moisture, may be the best metric for determining pelleted diet homogeneity and accordance to diet formulation.

REFERENCES AND NOTES

1. Creger, C. R. 1957. A study of distribution of micro-ingredients in mixed feeds. MS Thesis. Kansas State Univ., Manhattan.
2. McCoy, R. A., K. C. Behnke, J. D. Hancock, and R. R. McElhiney. 1994. Effect of mixing uniformity on broiler chick performance. *Poult. Sci.* 73:443-451.
3. Clark, P. M., K. C. Behnke, and D. R. Poole. 2007. Effects of marker selection and mix time on the coefficient of variation (mix uniformity) of broiler feed. *J. Appl. Poult. Res.* 16:464-470.
4. Pfost, H. B., C. Deyoe, C. Stevens, and E. Morgan. 1966. Testing feed mixtures, mixers and related equipment. *Feedstuffs* 38:32-46.
5. Beaman, K. R., K. G. S. Lilly, C. K. Gehring, P. J. Turk, and J. S. Moritz. 2012. Influence of pelleting on the efficacy of an exogenous enzyme cocktail using broiler performance and metabolism. *J. Appl. Poult. Res.* 21:744-756.
6. MFP Vertical Mixer, Easy Automation Inc., Welcome, MN.
7. Chloride QuanTab Test Strips, 30-600 mg/L, Hach, Loveland, CO.
8. AgriStats Inc., Fort Wayne, IN; 2013.
9. Master Model Pellet Mill, California Pellet Mills Company, Crawfordsville, IN.
10. Humboldt Mfg. Co., Elgin, IL.
11. Official Methods of Analysis of AOAC Int., (2000) 17th Ed., AOAC Int., Gaithersburg, MD, USA, Official Method 934.01.
12. Official Methods of Analysis of AOAC Int., (2000) 17th Ed., AOAC Int., Gaithersburg, MD, USA, Official Method 942.05.
13. Official Methods of Analysis of AOAC Int., (2000) 17th Ed., AOAC Int., Gaithersburg, MD, USA, Official Method 984.13.

14. Official Methods of Analysis of AOAC Int., (2000) 17th Ed., AOAC Int., Gaithersburg, MD, USA, Official Method 920.39.
15. Official Methods of Analysis of AOAC Int., (2000) 17th Ed., AOAC Int., Gaithersburg, MD, USA, Official Method 692.09.
16. Official Methods of Analysis of AOAC Int., (2000) 17th Ed., AOAC Int., Gaithersburg, MD, USA, Official Method 994.12.
17. New Jersey Feed Labs, Ewing Township, NJ.
18. The University of Missouri Agricultural Experiment Station Chemical Laboratories (ESCL), Columbia, MO.
19. SAS Institute. 2013. The SAS System for Windows 2000. Release 8.1. SAS Inst. Inc., Cary, NC.
20. Payne, J.D., Rattink, W., Smith, T., and Winowiski, T. 2001. Borregaard LignoTech. The Pelleting Handbook: A Guide for Production Staff in the Compound Feed Industry. 8.2.2:56-57.
21. Tang, P., P. H. Patterson, and V. M. Puri. 2006. Effect of feed segregation on the commercial hen and egg quality. J. Appl. Poult. Res. 15:564-573.
22. Moritz, J. 2013. Pellet quality performance tests: milling to bird performance. Proc. Arkansas Nutrition Conference. September 3 – 5. Rogers, AR.

Table 1: Selected Analyzed Nutrients¹ of Corn, Soybean Meal, Meat and Bone Meal, and Dried Distillers Grains and Solubles Used in the Diet Formulation.

Ingredients	Moisture (%)	Crude Protein (%)	Crude Fat (%)	Total Ash (%)
Corn	15.40	6.22	3.05	1.03
Soybean Meal	11.61	45.66	1.12	6.22
Porcine Derived M&B Meal	7.15	50.10	9.22	28.26
DDGS	13.61	26.58	7.52	4.80

¹Values obtained from New Jersey Feed Labs (Ewing Township, NJ)

Table 2: Ingredients and Calculated Nutrient Composition of the Broiler Finisher Diet

Ingredient	Inclusion, %
Corn	65.02
Soybean Meal (48%)	18.79
Meat and Bone Meal	5.00
Dried Distillers Grains and Solubles	5.00
Soybean Oil	3.22
Dicalcium Phosphate	1.24
Limestone	0.68
Sodium Chloride	0.28
Vitamin/Trace Mineral Premix ¹	0.25
DL-Methionine	0.21
L-Lysine HCL	0.19
L-Threonine	0.05
Sodium Bicarbonate	0.04
Ronozyme HiPhos ²	0.03
Calculated Nutrients³	
Metabolizable Energy (kcal/kg) ⁴	2993
Moisture (%)	15.00
Crude Protein (%) ⁵	16.49
Crude Fat (%)	6.11
Crude Fiber (%)	2.31
Total Ash (%)	5.54
Calcium (%)	1.03
Available Phosphorus (%) ⁴	0.54
Sodium (%)	0.19
Lysine (%)	0.87
Methionine + Cystine (%)	0.67
Threonine (%)	0.57

¹Supplied per kg of diet: manganese, 0.02%; zinc 0.02%; iron, 0.01%; copper, 0.0025%; iodine, 0.0003%; selenium, 0.00003%; folic acid, 0.69mg; choline, 386mg; riboflavin, 6.61mg; biotin, 0.03mg; vitamin B6, 1.38mg; niacin, 27.56mg; pantothenic acid, 6.61mg; thiamine, 2.20mg; manadione, 0.83mg; vitamin B12, 0.01mg; vitamin E, 16.53 IU; vitamin D3, 2133 ICU; vitamin A, 7716 IU

²DSM Nutritional Products- phytase.

³Values obtained from BRILL after nutrient specifications for corn, SBM, MBM, and DDGS were adjusted (Table 2) and after moisture was adjusted to 15%.

⁴Metabolizable Energy and Available Phosphorus were based on Agristat values as suggested by M. Donohue. 2013. The Challenges in Feeding Broilers in Times of High and Volatile Feed Ingredient Costs: How to Cover the Costs?. 2013 Mid-Atlantic Nutrition Conference proceedings. Calcium values were adjusted to a 2.1:1 of Calcium to Available phosphorus.

⁵Digestible amino acids were based on ratios to lysine suggested by P. B. Tillman and W.A. Dozier. 2013. Current Amino Acid Considerations for Broilers: Requirements, Ratios, Economics. www.thepoultryfederation.com for 8 – 14 day broilers. Digestible amino acid to digestible lysine ratios followed further recommendations of this communication (45 methionine, 70 threonine, 16 tryptophan, 105 arginine).

Table 3: Coefficient of Variation of a Corn and Salt Formulation and a Complete Corn and Soybean Based Formulation Mixed for 30 Seconds and 10 Minutes.

Corn-Salt ¹		
	30 Second Mix Time	10 Minute Mix Time
Mix 1	18.40%	9.10%
Mix 2	21.21%	7.30%
Mix 3	25.23%	6.59%
Mean Value	21.61	7.66
ANOVA Probability Value Comparing 30 Second to 10 Minute Mix Times	0.0028	
Complete Feed ²		
Mix 1	10.85 %	9.57 %
Mix 2	8.63 %	8.39 %
Mix 3	15.38 %	11.28 %
Mean Value	11.62	9.75
ANOVA Probability Value Comparing 30 Second to 10 Minute Mix Times	0.434	

¹Formulation containing corn and 3% sodium chloride and batched to 136 kg total.

²Complete feed formulation batched to 136 kg total and based on nutrient recommendations for broilers in the finisher phase.

Table 4: Main Effect Means and Probability Values for Main Effects and Interactions for Pelleted Subsample Nutrient Analyses.

2 x 2 x 2 x 2 factorial arrangement of treatments				Analyzed nutrients and feed ingredients				
				Crude Protein (%)	Crude Fat (%)	Total Ash (%)	Free supplemental DL-methionine (%)	Free supplemental L-lysine HCL (%)
Main Effect Means								
Mix Time Means								
30 Seconds	----	----	----	16.819	6.274	5.531	0.191	0.154
10 Minutes	----	----	----	16.828	6.112	5.668	0.197	0.155
Mix Time SEM ¹				0.122	0.036	0.050	0.002	0.002
Sampling Location Means								
----	Pellet Die²	----	----	16.891	6.242	5.620	0.196	0.155
----	Finished Feed³	----	----	16.755	6.145	5.578	0.191	0.153
Sample Location SEM				0.122	0.036	0.050	0.002	0.002
Sampling Number Means								
----	----	2 Samples	----	16.628	6.233	5.647	0.195	0.155
----	----	10 Samples	----	17.019	6.153	5.552	0.193	0.154
Sampling Number SEM				0.122	0.036	0.050	0.002	0.002
Blending Technique Means								
----	----	----	Hand Blending	16.838	6.203	5.597	0.195	0.154
----	----	----	Splitting Device	16.809	6.183	5.602	0.193	0.155
Blending Technique SEM				0.122	0.036	0.050	0.002	0.002
Probability Values for Mix Time x Sampling Location x Sampling Number x Blending Technique Factorial Arrangement								
Mix Time				0.9603	0.0030	0.0605	0.0195	0.5517
Sampling Location				0.4370	0.0623	0.5547	0.0408	0.3543
Sampling Number				0.0308	0.1231	0.1847	0.2747	0.6820
Blending Technique				0.8700	0.6970	0.9471	0.5519	0.6549
Sampling Location x Sampling Number				0.5302	0.8279	0.0313	0.7315	0.4802

¹Samples taken post-extrusion.²Samples taken at the sack-off bin after bagging.³SEM=Standard error of the mean.

CHAPTER 3: Efficacy of a novel protease provided to broiler chicks in diets that vary in composition and degree of processing

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SUMMARY Exogenous enzymes added at the mixer should demonstrate activity post-pelleting and improve bird performance to justify use in the broiler industry. Past research has shown that diet substrate and the pelleting process can effect enzyme efficacy. The objective of the study was to evaluate the feeding value of a novel protease in unprocessed mash and pelleted diets that were composed of all vegetable protein or an inclusion of animal protein. Treatments were arranged in a 2 x 2 x 2 factorial within a randomized complete block design. Protease inclusion was either 0 or 2%. Diet composition was either all-vegetable protein or an 8% porcine meat and bone meal inclusion. Diets were fed as unprocessed mash or ground pellets that were steam conditioned at 70°C and extruded through a 4 x 38 mm pellet die. Pellets were ground prior to feeding to eliminate potentially confounding feed form effects. All diets were formulated to be 85% of crude protein and digestible amino acid recommendations for young broilers. In addition, two unprocessed mash diets representing the all-vegetable protein or 8% porcine meat and bone meal inclusion were formulated to be 100% of crude protein and digestible amino acid recommendations and were fed as controls. Dietary treatments were fed to nine replicate raised wire cages of eight straight-run Hubbard x Cobb broiler chicks for 21 d. Protease activity was confirmed post pelleting and expressed in tyrosine releasing units that compare the absorbance of tyrosine liberated by protease digested casein to a standard curve generated by a tyrosine dilution. Protease and Degree of Processing interacted to effect chick weight gain and feed conversion ratio (FCR) ($P<0.05$). Protease applied to pelleted diets improved 21 d weight gain by 57g and decreased 21 d FCR by 0.07. Similar improvements were not observed in unprocessed mash diets. Linear contrasts clarified that protease applied to pelleted diets was most beneficial for all vegetable protein compositions ($P<0.05$). Diet Composition and Degree of Processing interacted to effect FCR ($P<0.05$). Meat and bone meal inclusion increased feed

conversion ratio in unprocessed mash diets but not pelleted diets. Protease efficacy was influenced by diet composition and degree of processing.

Keywords: Protease, Activity, Pelleting, Broiler, Performance

DESCRIPTION OF PROBLEM

Feed and feed manufacture account for the largest cost of broiler production. Profitability for the integrator and the grower can be dependent on multiple feed variables, such as ingredient cost, pelleting cost, and nutrient availability of ingredients post manufacture [1]. Nutritionists may be able to improve nutrient availability or decrease diet costs by incorporating exogenous enzymes in the formulation, such as phytase, xylanase, amylase, glucanase, or protease. Exogenous enzymes added at the mixer should demonstrate activity post-pelleting to show that conditions of heat, moisture, and pressure have not diminished the products potential to function. However, in vitro testing of enzyme activity post-pelleting per se may not predict efficacy in the animal [2-4]. Loop and coauthors [6] found that in vitro nutrient releasing assays for phytase do not always predict broiler chick performance benefits.

Sigma-Aldrich developed a non-specific activity assay for proteases [5]. In this particular assay, casein acts as the substrate for protease and liberation of the amino acid tyrosine is quantified through readings from a spectrophotometer. The readings express activity in tyrosine releasing units per gram (TU/g) based on a calculation comparing feed samples with and without protease. Research has also suggested that enzyme inactivation can occur due to frictional heat at the mash-die interface [7-9]. Gehring et al. [8] examined thermal stability of an exogenous enzyme cocktail (EEC) when added to diets containing either 1 or 4% mixer added fat (MAF). The authors concluded that diets containing an EEC with 4% MAF decreased relative electrical energy usage of the pellet mill (PREEU), improved live weight gain, and decreased FCR. Another experiment performed by Corey et al. [9] demonstrated that increasing MAF to 3% in a diet containing 0.5% calcium lignosulfonate decreased FCR and improved true amino acid digestibility (TAAD). These findings suggest that increasing MAF lubricates the pellet die,

decreases frictional force, improves nutrient digestibility, and may enable improved exogenous enzyme survivability [8, 9].

Two experiments were conducted utilizing a novel plant based protease granulated with zeolite and designed to be added to feed at the mixer [10]. The objective of the first experiment was to determine protease activity using a tyrosine releasing laboratory assay and develop an appropriate diet formulation and conditions of feed manufacture to subsequently be used for broiler feeding. The objective of the second experiment was to evaluate the feeding effects of the protease in unprocessed mash and pelleted diets that were composed of all vegetable protein or an 8% inclusion of porcine meat and bone on broiler performance.

MATERIALS AND METHODS

Experiment 1

Protease Assay

A feed manufacture experiment was performed to determine proper conditioning temperature and protease inclusion for broiler diets. *In vitro* general protease activity assays were utilized and based on methods developed by Sigma-Aldrich [5]. Assays were performed on the pure protease, mash, and pelleted feed samples with and without protease. Either 0.1 g pure protease or 2.0 g feed samples were weighed and placed into 250mL Erlenmeyer flasks. One hundred mL of an assay buffer (sodium phosphate, EDTA, and Cysteine-HCl solution, pH 6.0) was added to each flask and mixed for 10 minutes. During sample mixing, five mL of a casein solution was added to round bottom tubes (two for each protease/feed sample), covered with Parafilm, and placed in a 40°C water bath for 10 minutes. Tubes were removed from the water

bath, two mL from Erlenmeyer flasks were added, and tubes were again covered with Parafilm and placed back into the water bath for 60 minutes. Once again tubes were removed from the water bath, 3 mL 30% trichloroacetic acid was added to each tube, and tubes were covered with Parafilm and placed back into the water bath for 30 minutes. Tubes were removed and centrifuged for 5 minutes at 13,000 rpm. Supernatant was poured into a 15 mL tube and read on a spectrophotometer at 280° absorbance (Abs.). Readings were included in the following equation to calculate tyrosine releasing units per gram:

$$\text{TU/g Feed} = \frac{((\text{Sample Abs.} - \text{Blank Abs.}) \times \text{Standard Curve Slope})}{60} \times \frac{100\text{mL}}{\text{Wt.}} \times \frac{10\text{mL}}{2\text{mL}}$$

Feed samples that contained protease were then corrected to eliminate error associated with tyrosine release in feed without protease.

$$\text{Corrected TU/g Feed} = \text{TU/g Diet Containing Protease} - \text{TU/g Diet Containing No Protease}$$

Enzyme activity per se TU/g was then calculated using the following calculation:

$$\text{TU/g Enz} = \frac{(\text{TU/g Diet Containing Protease} - \text{TU/g Diet Containing No Protease})}{\% \text{ Protease Inclusion}}$$

Feed Manufacture

Based on general inclusions of commercially available exogenous enzymes, a corn and soybean meal based diet was formulated to contain 0.03% protease and 1.82% MAF. Feed was manufactured using a 40 HP California Pellet Mill [11] and steam conditioned at 50, 60, or 70°C for ten seconds before passing through a 4 x 38 mm pellet die. Ten mash samples per treatment were taken from the mixer [12] and three hot pellet samples per treatment were taken post-extrusion from the pellet die. This sampling area was previously found to provide the best representation of calculated nutrients and ingredient inclusion of diets [13]. Hot pellet samples

were cooled using ambient air and a fan for 12 minutes. Samples were analyzed before and after pelleting to determine thermal stability of the protease. Analysis of TU/g could not differentiate between samples with or without protease likely due to protease inclusion being too low for the sensitivity of the activity assay within a complete feed. Diets were then reformulated to contain either 1, 2, 5, or 10% protease and activity assays were conducted on unprocessed mash samples. A diet containing 2% protease and 1.82% MAF was formulated and steam conditioned at 50, 60, 70, and 80°C for ten seconds and extruded through a 4 x 38 mm pellet die. Subsequently, a diet containing 2% protease and 4.25% MAF was formulated and steam conditioned at 50, 60, 70, and 80°C for ten seconds and extruded through a 4 x 38 mm pellet die. Samples were collected and analyzed as previously described. These experiments were preliminary and pelleted batches were not replicated. Therefore these data should be viewed as descriptive.

Experiment 2

Feed Manufacture

Corn and soybean meal based diets were formulated to contain either all vegetable protein or an 8% inclusion of porcine derived meat and bone meal (M&B) (Table 1). Protease inclusion was either 0 or 2%. Diets were fed as unprocessed mash or ground pellets that were steam conditioned at 70°C for 10 seconds and extruded through a 4 x 38 mm pellet die. Pellets were ground prior to feeding to eliminate potentially confounding feed form effects. All diets were formulated to be 85% of crude protein and digestible amino acid recommendations for young broilers [14]. In addition, two unprocessed mash diets representing the all vegetable protein or an 8% inclusion of porcine meat and bone meal were formulated to be 100% of crude protein and digestible amino acid recommendations and were fed as controls.

All feed was manufactured at the West Virginia University Pilot Feed Mill. Each experimental diet was batched and split into two, 113 kg allotments. One allotment of each diet was fed as unprocessed mash and one allotment was conditioned and pelleted. Cooled pellets were passed through a roller mill to create a feed particle size similar to that of a mash diet to eliminate potentially confounding feed form effects (table 4). Pelleted diet samples were taken post-extrusion from the pellet die and immediately cooled using ambient air and a fan for 12 minutes. Samples were used to determine particle size [14] (Table 4) and protease activity post pelleting (Table 5).

Live Bird Performance

A total of 720 Hubbard x Cobb 500 straight-run broiler chicks obtained from a commercial hatchery [16] were reared in raised wire cages (30.48 x 50.80 cm) in two cross-ventilated, negative-pressure rooms that contained 48 cages each. Each dietary treatment was fed to nine replicate pens containing eight broilers/cage. The experimental unit was considered one cage. Blocking was conducted based on adjacent cage area within the rooms. Each cage contained nipple drinkers and an external feed trough. Mortalities from d 1-3 were replaced. Birds were weighed as an experimental unit on d 1 and 21. On d 18, 0.2% titanium dioxide was added to the feed via top-dress. On d 21, five randomly selected birds/cage were euthanized via cervical dislocation, weighed, and the lower ileum was excised and gently flushed to remove digesta. Measured variables included feed intake per bird (FI), live weight gain per bird (LWG), ending bird weight (EBW), and mortality corrected FCR. All birds were reared using environmental recommendations obtained from Cobb-Vantress [17], and approved protocols

were established by the West Virginia University Animal Care and Use Committee [ACUC 15-0408].

Statistical Analysis

A 2 x 2 x 2 factorial arrangement in a randomized complete block design was utilized to explore the main effects and interactions of diet substrate, feed processing, and protease inclusion. The experimental unit was one pen containing eight broilers. Blocking criterion consisted of cage location in the rooms. Analyses were completed using the GLM ANOVA procedure from the SAS Institute. When main effects significantly interacted, treatments were compared using Fisher's LSD multiple comparison test. Linear contrasts were performed to clarify main effect interactions. Means were considered significantly different at $\alpha \leq 0.05$.

RESULTS AND DISCUSSION

Experiment 1

Feed samples that contained 0.03% protease were indistinguishable from feed samples that did not contain protease in terms of TU/g (data not shown). Therefore, diets were reformulated to contain either 1, 2, 5, or 10%, protease and activity assays were conducted on unprocessed mash samples (Table 2). The 2% protease level was chosen for continued testing in conditioning and pelleting survivability due those formulations generating repeatable and relatively high in vitro activity. A diet containing 2% protease and 1.82% MAF was then steam conditioned at 50, 60, 70, and 80°C. However, protease assays demonstrated low activity (data

not shown). Hot pellet temperature (HPT), generated during pelleting of these diets was elevated by approximately 20°C for all conditioning temperatures. Suspecting that MAF was too low for enzyme survivability, a new diet was formulated containing 2% protease and 4.25% MAF. Protease activity was retained for each conditioning temperature and decreased as conditioning temperature increased (Table 3). A similar result was found by Gehring et al. [8], who tested thermal stability of an EEC when added to diets containing either 1 or 4% MAF. The authors found that diets containing the EEC with 4% MAF decreased relative electrical energy usage of the pellet mill (PREEU), improved live weight gain, and decreased FCR of subsequently fed chicks. These results were likely associated with increased lubrication at the mash-die interface and decreased frictional heat generation. A conditioning temperature of 70°C was selected for further testing in Experiment 2 due to that temperature being closer to commercial feed manufacture practices, while maintaining activity that we speculated could still produce an in vivo response.

Experiment 2

Descriptive results of the physical characteristic tests of pelleted feed are found in Table 4. Pellet durability among treatments was not remarkable and particle size analysis demonstrated that pellet grinding had the effect of producing a similar particle sized feed as unprocessed mash treatments.

Protease recovery data is shown in table 5. Unprocessed mash diets that contained 8% porcine meat and bone meal (M&B) numerically decreased protease activity compared to all-vegetable diets. Furthermore, no activity was detected for the protease when applied to diets that contained 8% M&B and were pelleted. In regard to live performance, protease and degree of

processing interacted to effect chick weight gain and feed conversion ratio (FCR) ($P < 0.05$). Protease applied to pelleted diets improved 21 d weight gain by 57g and decreased 21 d FCR by 0.07. Similar improvements were not observed in unprocessed mash diets. Linear contrasts clarified that protease applied to pelleted diets was most beneficial for all vegetable protein compositions ($P < 0.05$). The particular protease likely has a greater effect on vegetable proteins compared to animal proteins. The diet containing 8% porcine meat and bone meal had a reduced soybean meal content of approximately 30% compared to the all-vegetable protein diet. The reduced protease activity due to the conditioning and pelleting process likely further reduced enzyme efficacy in the 8% porcine meat and bone meal diets. Evans and Moritz [22] observed similar results when broilers were fed diets that contained either vegetable or animal protein and a protease. Birds that were fed all vegetable protein diets with protease had greater amino acid digestibility, increased BWG, and improved FCR compared to birds that were fed diets containing animal protein with protease [22]. In regard to improved protease efficacy with diets that were pelleted, perhaps the soybean meal substrate underwent conformational changes due to pelleting that improved protease accessibility. Similar results have been observed for the effect of glucanase and pelleted barley based diets [23].

Diet Composition and Degree of Processing interacted to effect FCR ($P < 0.05$). Meat and bone meal inclusion increased feed conversion ratio in unprocessed mash diets but not pelleted diets. The conditioning and pelleting process may have improved digestibility of the porcine meat and bone meal. Past research has shown benefits of protease inclusion in broiler diets. Keratinase added to corn and soybean meal based broiler diets formulated to be 93% of NRC requirement for crude protein has been shown to increase bird ending body weight up to 32 g [18]. Stark et al. [19] also utilized keratinase in corn and soybean meal based broiler diets and

performed enzymatic recovery assays. Despite decreased activity retention in pelleted diets (66%) compared to mash diets (93%), inclusion of keratinase in pelleted diets increased weight gain (798 to 838g) and improved FCR (1.57 to 1.55) relative to diets without keratinase. The conditions of the current study suggest that protease efficacy was influenced by diet composition and degree of processing.

CONCLUSIONS AND APPLICATIONS

1. *In vitro* protease activity was demonstrated for all diets through tyrosine releasing units except for pelleted diets containing 8% porcine derived meat and bone meal.
2. Feed Processing x Protease Inclusion interactions showed improvement in broiler EBW, LWG, and FCR for broilers that were fed diets that were pelleted and contained protease.
3. Performance improvements were more prominent in diets containing all vegetable protein.

REFERENCES AND NOTES

1. Beaman, K. R., K. G. S. Lilly, C. K. Gehring, P. J. Turk, and J. S. Moritz. 2012. Influence of pelleting on the efficacy of an exogenous enzyme cocktail using broiler performance and metabolism. *J. Appl. Poult. Res.* 21:744-756.
2. Malathi, V. and G. Devegowda. 2001. In vitro evaluation on nonstarch polysaccharide digestibility of feed ingredients by enzymes. *Poult. Sci.* 80:302-305.
3. Freitas, D. M., S. L. Vieira, C. R. Angel, A. Favero, and A. Maiorka. 2011. Performance and nutrient utilization of broilers fed diets supplemented with a novel mono-component protease. *J. Appl. Poult. Res.* 20:322-334.
4. Loop, S. A., K. G. S. Lilly, L. K. Shires, C. K. Gehring, K. R. Beaman, M. E. Persia, and J. S. Moritz. 2012. The phytase analytical activity of pelleted diets may not adequately describe efficacy in the bird. *J. Appl. Poult. Res.* 21:492-501.
5. Cupp-Enyard, C. 2008. Sigma's non-specific protease activity assay – casein as a substrate. *J. Vis. Exp.* 19:889.

6. Angel, C. R., W. Saylor, S. L. Vieira, and N. Ward. 2011. Effects of a monocomponent protease on performance and protein utilization in 7- to 22-day-old broiler chickens. *Poult. Sci.* 90:2281-2286.
7. Lamp, A. E., A. M. Evans, and J. S. Moritz. 2015. The effects of pelleting and glucanase supplementation in hulled barley based diets on feed manufacture, broiler performance, and digesta viscosity. *J. Appl. Poult. Res.* 24:295-303.
8. Gehring, C. K., K. G. S. Lilly, L. K. Shires, K. R. Beaman, S. A. Loop, and J. S. Moritz. 2011. Increasing mixer-added fat reduces the electrical energy required for pelleting and improves exogenous enzyme efficacy for broilers. *J. Appl. Poult. Res.* 20:75-89.
9. Corey, A. M., K. G. S. Wamsley, T. S. Winowiski, and J. S. Moritz. 2014. Effects of calcium lignosulfonate, mixer-added fat, and feed form on feed manufacture and broiler performance. *J. Appl. Poult. Res.* 23:418-428.
10. Kiwi Enzyme, LLC. Auckland, New Zealand.
11. Master Model Pellet Mill, California Pellet Mills Company, Crawfordsville, IN.
12. MFP Vertical Mixer, Easy Automation Inc., Welcome, MN.
13. Reese, D. A., K. L. Foltz, and J. S. Moritz. 2015. Effect of feed mixing and sampling variables on nutrient analysis. *Poult. Sci.* (Accepted Abstract (101)).
14. Tillman, P. B. and W. A. Dozier, III. 2013. Update on Amino Acids for Broilers. The Poultry Federation.
15. RO-Tap RX-29, W.S. Tyler, Mentor, OH.
16. Pilgrim's Pride Hatchery, Moorefield, WV.
17. Cobb-Vantress, Siloam Springs, AR. 2008. Cobb 500 Broiler Performance and Nutrition Supplement.
18. Odetallah, N. H., J. J. Wang, J. D. Garlich, and J. C. H. Shih. 2003. Keratinase in starter diets improves growth of broiler chicks. *Poult. Sci.* 82:664-670.
19. Stark, C. R., B. E. Spencer, J. C. H. Shih, C. G. Chewning, and J. J. Wang. 2009. Evaluation of keratinase stability in pelleted broiler diets. *J. Appl. Poult. Res.* 18:30-33.
20. Loop, S. A., K. G. S. Lilly, L. K. Shires, C. K. Gehring, K. R. Beaman, M. E. Persia, and J. S. Moritz. 2012. The phytase analytical activity of pelleted diets may not adequately describe efficacy in the bird. *J. Appl. Poult. Res.* 21:492-501.

21. Corey, A. M., K. G. S. Wamsley, T. S. Winowiski, and J. S. Moritz. 2014. Effects of calcium lignosulfonate, mixer-added fat, and feed form on feed manufacture and broiler performance. *J. Appl. Poult. Res.* 23:418-428.
22. Evans, A. M. and J. S. Moritz. 2014. Effects of dietary enzyme supplementation on nutrient digestibility and growth performance of chicks fed diets with high amounts of meat and bone meal. *Poult. Sci.* (Accepted Abstract (60)).
23. Lamp, A. E., A. M. Evans, and J. S. Moritz. 2015. The effects of pelleting and glucanase supplementation in hulled barley based diets on feed manufacture, broiler performance, and digesta viscosity. *J. Appl. Poult. Res.* 24:295-303.

Table 1: Ingredients and Calculated Nutrient Composition of Broiler Starter Diets

Ingredient	Inclusion (%) Vegetable Protein, 85% Digestible AA¹	Inclusion (%) M&B Meal, 85% Digestible AA²	Inclusion (%) Vegetable Protein, 100% Digestible AA³	Inclusion (%) M&B Meal, 100% Digestible AA⁴
Corn	54.21	59.31	44.54	49.60
Soybean Meal	34.94	25.53	43.47	34.19
Porcine Meat & Bone Meal	-	8.00	-	8.00
Soybean Oil	4.25	3.23	5.33	4.30
Dicalcium Phosphate	2.09	0.34	2.03	0.28
Sand or Protease	2.00	2.00	2.00	2.00
Limestone	1.48	0.50	1.46	0.48
DL-Methionine	0.30	0.32	0.41	0.40
Salt	0.29	0.15	0.30	0.15
Poultry Vitamin Premix ⁵	0.25	0.25	0.25	0.25
Sodium Bicarbonate	0.15	0.15	0.15	0.15
Lysine	-	0.12	-	0.09
L-Threonine	0.04	0.10	0.06	0.11
Total	100	100	100	100
Calculated Nutrients				
Metabolizable Energy (Kcal/kg) ⁶	2,999.64	2,999.64	2,999.64	2,999.64
Crude Protein ⁷	20.40	20.40	24.00	24.00
Calcium	1.08	1.08	1.08	1.08
Available Phosphorus ⁶	0.51	0.51	0.51	0.51
Sodium	0.17	0.17	0.17	0.17
Digestible Lysine	1.02	1.02	1.22	1.20
Digestible Methionine	0.43	0.46	0.52	0.53
Digestible Methionine + Cystine	0.93	0.93	1.09	1.06
Digestible Threonine	0.71	0.71	0.84	0.84

¹Diet formulation utilizing vegetable protein and containing 85% digestible crude protein and amino acids. Formulation was used for the following four experimental diets: Diet 1- Mash, Sand. Diet 2- Ground Pellet, Sand. Diet 3- Mash, Actinidin. Diet 4- Ground Pellet, Actinidin.

²Diet formulation utilizing 8% porcine meat and bone meal and 85% digestible crude protein and amino acids. Formulation was used for the following four experimental diets: Diet 5- Mash, Sand. Diet 6- Ground Pellet, Sand. Diet 7- Mash, Actinidin. Diet 8- Ground Pellet, Actinidin.

³Diet formulation utilizing vegetable protein and containing 100% digestible crude protein and amino acids. Formulation was used for the following control diet: Diet 9- Mash, Sand.

⁴Diet formulation utilizing 8% porcine meat and bone meal and 100% digestible crude protein and amino acids. Formulation was used for the following control diet: Diet 10- Mash, Sand.

⁵Supplied per kilogram of diet: 0.02% manganese; 0.02% zinc; 0.01% iron; 0.0025% copper; 0.0003% iodine; 0.00003% selenium; 0.69 mg of folic acid; 386 mg of choline; 6.61 mg of riboflavin; 0.03 mg of biotin; 1.38 mg of vitamin B6; 27.56 mg of niacin; 6.61 mg of pantothenic acid; 2.20 mg of thiamine; 0.83 mg of menadione; 0.01 mg of vitamin B12; 16.53 IU of vitamin E; 2,133 ICU of vitamin D3; and 7,716 IU of vitamin A.

⁶Metabolizable Energy and Available Phosphorus were based on Agristat values as suggested by M. Donohue. 2013. The Challenges in Feeding Broilers in Times of High and Volatile Feed Ingredient Costs: How to Cover the Costs?. 2013 Mid-Atlantic Nutrition Conference proceedings. Calcium values were adjusted to a 2.1:1 of Calcium to Available phosphorus.

⁷Digestible amino acid reductions (15%) were based on digestible amino acid ratios to lysine suggested by P. B. Tillman and W.A. Dozier. 2013. Current Amino Acid Considerations for Broilers: Requirements, Ratios, Economics. www.thepoultryfederation.com for 8 – 14 day broilers. Digestible amino acid to digestible lysine ratios followed further recommendations of this communication (45 methionine, 70 threonine, 16 tryptophan, 105 arginine).

Table 2: Determination of Dosage of Protease to be Utilized in Broiler Diets Using Unprocessed Mash¹

Sample	TU/g Feed ²	Corrected ³ (TU/g)	TU/g Enz. ⁴
Pure Protease	-	-	6,890
Feed + 0% Protease	217.93	-	-
Feed + 1% Protease	254.63	36.70	3,670
Feed + 0% Protease	198.21	-	-
Feed + 2% Protease	287.49	89.28	4,464
Feed + 0% Protease	266.48	-	-
Feed + 5 % Protease	417.29	150.81	3,016
Feed + 0% Protease	191.33	-	-
Feed + 10% Protease	613.65	422.32	4,223

¹Corn and soybean meal based diet with 1.82% MAF.

²Tyrosine Releasing Units/g of tyrosine liberated from the feed using the TU Assay = ((Sample Abs. – Blank Abs.) x Standard Curve Slope)/60 x 100mL/Wt. x 10mL/2mL

³Removal of error from tyrosine present in feed per se = TU/g Diet Containing Protease – TU/g Diet Containing No Protease

⁴Tyrosine Releasing Units per gram of enzyme = (TU/g Diet Containing Protease – TU/g Diet Containing No Protease) / % Protease Inclusion

Table 3: Determination of Conditioning Temperature for 2% Inclusion of Protease in an All Vegetable Protein Diet¹

Feed Processing	TU/g Feed ²	Corrected ³ (TU/g)	TU/g Enz. ⁴	Retention (%) ⁵
2% Inclusion Mash ⁶	227.06	53.94	2697.36	-
50°C	281.32	54.26	2712.90	100%
60°C	269.31	42.25	2112.35	78%
70°C	250.14	23.08	1153.72	43%
80°C	241.22	14.16	707.59	26%

¹Pelleted corn and soybean meal based diets with 4.25% MAF were steam conditioned for 10 seconds.

²Tyrosine Releasing Units/g of tyrosine liberated from the feed using the TU Assay = ((Sample Abs. – Blank Abs.) x Standard Curve Slope)/60 x 100mL/Wt. x 10mL/2mL

³Removal of error from tyrosine present in feed per se = TU/g Diet Containing Protease – TU/g Diet Containing No Protease

⁴Tyrosine Releasing Units/g of tyrosine liberated from the protease alone = (TU/g Diet Containing Protease – TU/g Diet Containing No Protease) / % Protease Inclusion

⁵Amount of enzymatic activity retained in pelleted diets.

⁶2% inclusion of protease in unprocessed mash samples.

Table 4: Descriptive Feed Manufacture Data of Experiment 2

Diet Substrate	Feed Processing	Novel Protease Inclusion	PDI (%)¹	MPDI (%)²	New Holman (%)³	Particle Size (μ)⁴
All Vegetable	Ground Pellet	No	76.81	65.77	48.26	995.51
		Yes	79.80	72.47	52.81	937.89
	Unprocessed Mash	No	-	-	-	955.78
		Yes	-	-	-	985.50
	Unprocessed Mash PC	No	-	-	-	957.56
Meat and Bone Meal Inclusion	Ground Pellet	No	71.93	63.37	38.92	972.78
		Yes	74.59	65.70	40.92	964.25
	Unprocessed Mash	No	-	-	-	941.12
		Yes	-	-	-	941.07
	Unprocessed Mash PC	No	-	-	-	947.77

¹Pellet durability index obtained using a pfast tumbling box.

²Modified pellet durability index obtained using a pfast tumbling box and adding five hex nuts to the sample.

³Pellet durability index obtained using a New Holman Pellet Tester.

⁴Size of feed particles obtained using a ro-tap sieve shaker.

Table 5: Protease Activity Obtained From Experiment 2 Using the Tyrosine Releasing Unit Assay

Diet Substrate	Feed Processing	Novel Protease Inclusion	Tyrosine Releasing Units/g Feed¹	Corrected² (Tyrosine Releasing Units/g)	Tyrosine Releasing Units/g Enzyme³
All Vegetable	Ground Pellet	No	302.51	-	-
		Yes	408.31	75.62	3780.82
	Unprocessed Mash	No	312.24	-	-
		Yes	420.62	87.96	4398.07
	Unprocessed Mash PC	No	332.69	-	-
Meat and Bone Meal Inclusion	Ground Pellet	No	434.28	-	-
		Yes	415.14	-12.33	-616.66
	Unprocessed Mash	No	370.34	-	-
		Yes	481.35	53.89	2694.05
	Unprocessed Mash PC	No	427.47	-	-

¹Tyrosine Releasing Units/g of tyrosine liberated from the feed using the TU Assay = ((Sample Abs. – Blank Abs.) x Standard Curve Slope)/60 x 100mL/Wt. x 10mL/2mL

²Removal of error from tyrosine present in feed per se = TU/g Diet Containing Protease – TU/g Diet Containing No Protease

³Tyrosine Releasing Units/g of tyrosine liberated from the protease alone = (TU/g Diet Containing Protease – TU/g Diet Containing No Protease) / % Protease Inclusion

Table 6: Effects of a Novel Protease Included in Diets that Differ in Substrate and Processing on Broiler 0-21 Day Performance

Diet Substrate	Feed Processing	Novel Protease Inclusion	Bird Feed Intake (kg)	Bird Ending Weight (kg)	Bird Live Weight Gain (kg)	Feed Conversion Ratio (%)
All Vegetable ¹	Ground Pellet ³	No	1.027	0.779 ^d	0.732 ^d	1.43 ^a
		Yes ⁶	1.140	0.878 ^a	0.832 ^a	1.34 ^{def}
	Unprocessed Mash ⁴	No	1.069	0.859 ^{abc}	0.813 ^{ab}	1.32 ^{ef}
		Yes	1.053	0.816 ^{bcd}	0.770 ^{bcd}	1.37 ^{bcde}
	Unprocessed Mash PC ⁵	No	1.082	0.848 ^{abc}	0.802 ^{abc}	1.35 ^{cdef}
Meat and Bone Meal Inclusion ²	Ground Pellet	No	1.090	0.826 ^{abcd}	0.780 ^{abcd}	1.37 ^{abcd}
		Yes	1.079	0.839 ^{abc}	0.794 ^{abc}	1.33 ^{def}
	Unprocessed Mash	No	1.112	0.821 ^{abcd}	0.774 ^{abcd}	1.40 ^{abc}
		Yes	1.064	0.798 ^{cd}	0.752 ^{cd}	1.41 ^{ab}
	Unprocessed Mash PC	No	1.072	0.870 ^{ab}	0.824 ^{ab}	1.30 ^f
Probability Values for the Comprehensive Treatment Comparison						
ANOVA Probability Value			0.3616	0.0343	0.0325	0.0001
Fisher's LSD value ⁷			--	0.0607	0.0607	0.0553
Treatment SEM ⁸			0.0294	0.0215	0.0215	0.0196
Diet Substrate Means						
All Vegetable	----	----	1.072	0.833	0.787	1.36
Meat and Bone	----	----	1.086	0.821	0.775	1.38
Diet Substrate SEM			0.0161	0.0133	0.0133	0.0093
Feed Processing Means						
----	Ground Pellet	----	1.084	0.831	0.785	1.37
----	Unprocessed Mash	----	1.075	0.823	0.777	1.37
Feed Processing SEM			0.0161	0.0133	0.0133	0.0093
Protease Inclusion Means						
----	----	No	1.074	0.821	0.775	1.38
----	----	Yes	1.084	0.833	0.787	1.36
Protease Inclusion SEM			0.0161	0.0133	0.0133	0.0093
Probability Values for the Diet Substrate x Feed Processing x Protease Inclusion Factorial Treatment Arrangement						
Diet Substrate			0.5427	0.5316	0.5321	0.1652
Feed Processing			0.6817	0.7005	0.6948	0.5982
Protease Inclusion			0.6657	0.5296	0.5206	0.2589
Substrate * Processing			0.5605	0.3947	0.3869	0.0008
Substrate * Protease			0.0931	0.3882	0.3853	0.7952
Processing * Protease			0.0732	0.0204	0.0199	0.0004
Substrate * Processing * Protease			0.3212	0.1589	0.1555	0.1070
Probability Values for linear contrasts on specific treatment comparisons of interest						
Vegetable based ground pellet (protease vs. no protease)			0.0082	0.0017	0.0015	0.0019
Meat and Bone Inclusion based ground pellet (protease vs. no protease)			0.8063	0.6603	0.6574	0.1626

¹All Vegetable refers to a corn and soybean meal based diet,²Meat and Bone Meal Inclusion refers to an 8% inclusion to a corn and soybean meal based diet³Feed was steam conditioned at 70°C, extruded through a 38mm pellet die and ground to eliminate potentially confounding feed form effects on broiler performance. These diets were formulated to be 85% of crude protein and digestible amino acid nutrient recommendations.⁴Feed was not steam conditioned or pelleted. These diets were formulated to be 85% of crude protein and digestible amino acid nutrient recommendations.⁵PC refers to positive control diet formulations. These diets were formulated to be 100% of crude protein and digestible amino acid nutrient recommendations.⁶The protease was included at 2% of the diet formulation and added at the mixer prior to pelleting when applicable.⁷Fisher's least significant difference values.⁸Standard error of the mean.

CIRRICULUM VITAE

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Confident with the challenges associated with working under pressure. Confident with leading a group, working within a team, or as an individual.

Goals: To continue gaining knowledge in the poultry industry throughout my future career. I plan to use this knowledge to assist integrators in efficiently supplying protein to consumers.

EDUCATION

Degree obtained: Bachelor of Science in Agriculture, Animal and Nutritional Science; May 2014

Udergraduate GPA: 2.99

Expected Degree: Master of Science, Nutritional and Food Science

Expected Graduation Date: May, 2016

Current GPA: 2.73

EDUCATION HONORS/AWARDS

Graduated from Philipsburg-Osceola Area High School in 2010

Grants Recieved

- Pennsylvania Grant: 2010-2014
- Federal PELL Grant: 2011-2014

Awards and Honors

- Davis College of Agriculture, Natural Resources, and Design Dean's List
 - Fall 2013
-

First Author Publications

Abstracts

D.A. Reese, K.L. Foltz, and J.S. Moritz. 2015. Effect of feed mixing and sampling variables on nutrient analysis. Poult. Sci. (Accepted Abstract (101)).

D.A. Reese and J.S. Moritz. 2016. Efficacy of a novel protease provided to broiler chicks in diets that vary in composition and degree of processing. Poult. Sci. (Accepted Abstract).

RESEARCH EXPERIENCE

National Meeting Paper Presentations

- 2015 Poultry Science Association (Louisville, KY) (Graduate Student)
 - "Effect of feed mixing and sampling variables on nutrient analysis."

Graduate Teaching Assistant

- Teaching Assistant for Applied Nutrition II
 - Roles included instructing classes, grading exams, and assisting students in preparation for their research projects. I assisted in the proximate analysis, feed manufacture, chick placement, and data collection processes.

Graduate Research Assistant

- Led a study utilizing various mixing and sampling methodologies to develop a proper sampling protocol (November 2014)
- Led a study utilizing a protease enzyme product that was included in varying inclusions in diets that varied in ingredient composition and degree of processing (January 2015 – February 2016)
- Attended the National Poultry Science Association annual meeting
 - 2014- Corpus Christi, TX
 - 2015- Louisville, KY
- Led numerous WVU Animal Science Farm tours
 - WVU Residence Hall (Fieldcrest Hall)
 - Principles of Animal Science Class
 - Charleston, WV 5th grade class
 - Doddridge County FFA chapter
- International Production and Processing Exposition, Atlanta, GA
 - 2015, 2016
- Hands-on Ag Day- 2014, 2015
 - North Marion High School
- Poultry Festival (Moorefield, WV) (2014)

- Assisted with conducting an annual poultry judging competition
- Family Farm Day poultry display (2014, 2015)
- West Virginia State FFA Poultry CDE Competition (2014)
 - Created classes and coordinated the competitions
- Organic Field Day (2015)
 - Prepare/serve food
- Davis College Welcome Back BBQ (2014, 2015)
 - Prepare/serve food

EXPERIENCE

Pizza Hut	2013-2014
<ul style="list-style-type: none"> • Server/Cook/Driver 	
Euro Suites	2012-2013
<ul style="list-style-type: none"> • Housekeeper 	
WVU Dining Services	2011-2013
<ul style="list-style-type: none"> • Food Service Worker • Lead small groups • Prepared food 	
Best Western	2011
<ul style="list-style-type: none"> • Temporary/Summer Employment • Housekeeper 	
K-Mart	2011
<ul style="list-style-type: none"> • Temporary/College Employment • Deli counter 	

SKILLS

- Feed manufacture
- Pellet durability analysis
- Diet formulation
- Cecectomy surgery
- Tibia extraction
- Ileum extraction
- Breast extraction
- Enzymatic laboratory assays
- Ether extraction using Soxhlet apparatus
- Digesta viscosity analysis

- Poultry judging
- Proficient in Window's Microsoft Programs
- Agricultural/Animal Specific Undergraduate courses taken:
 - Intro to Animal Science 150
 - Principles of Animal Science 251
 - Animal Nutrition 260
 - Companion Animal Science 275
 - Equine Management and Training 281
 - Poultry Evaluation 338
 - Beef Production 341
 - Pork Production 353
 - Applied Nutrition 1 & 2/Lab 361&362/363
 - Poultry Production/Lab 367/369
 - Dairy Heifer Management 411

Graduate Courses:

- Food Animal Diseases 493C
- Introduction to Biochemistry 410
- Statistics 511 & 512
- Agriculture and Natural Resources Communicants 421
- Grants and Grantsmanship 593
- Nutritional Biochemistry 512
- Food Microbiology 545