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Evaluating the Effects of Modifying Mixing and Pelleting Parameters on Feed Quality, Pellet Production Rate, and Broiler Performance

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Evaluating the Effects of Modifying Mixing and Pelleting Parameters on Feed Quality, Pellet Production Rate, and Broiler Performance

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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 Effects of Modifying Mixing and Pelleting Parameters on Feed Quality, Pellet Production Rate, and Broiler Performance

Lucas E. Knarr

In Chapter 2, an experiment was conducted to evaluate the effect of different mixer types (**MTY**) and mix times (**MTI**) on mix uniformity (**MU**). A 2 (mix time) x 3 (mixer type) factorial was utilized in this experiment to create six treatments, each mixed in three randomized complete blocks. Mix times were designated adequate or inadequate, were based on the manufacturers' recommendation, and varied based on the mixer utilized. The mixer types utilized included a vertical mixer, horizontal mixer, and transfer mixing system. The transfer mixing system can be described as a batch-to-mix system that simulates the process of conveying batched feed to a mixing location. Chloride ions (**Cl^-**) via salt, crystalline DL-Methionine (**Free Met**), and crystalline L-Lysine-HCL (**Free Lys**) were utilized as coefficient of variation (**CV**) markers, with Cl^- being used in a corn-salt (**CS**) ration and the Free Met and Free Lys being used in a complete diet (**CD**). An interaction between MTY and MTI affected Free Met CV ($P = 0.005$). The horizontal mixer and transfer mixing system did not demonstrate changes in the Free Met CV compared to the vertical mixer when MTI was manipulated. The authors hypothesize that additional mixing during load-out and added conveyance caused this interaction. The transfer mixing system resulted in the lowest Free Lys CV ($P = 0.017$); however, no treatment provided an industry-acceptable MU. In conclusion, Free Lys may not be an appropriate marker for MU, Free Met may be an appropriate marker for MU in complete diets, and the transfer mixing system may contribute to an acceptable MU.

In Chapter 3, an experiment was conducted to evaluate the influence of pellet die thickness (**PDT**) and Azomite® (**AZM**) on feed manufacture. A 2 (AZM) x 3 (PDT) factorial was utilized in this experiment to create a total of six treatments, each manufactured in every run order position in a Latin Square Design. Azomite inclusion rate was either 0.0% (control) or 0.25%, and 32mm, 38mm, and 45mm PDTs were utilized in their appropriate treatments. The pellet diameter was held to a constant of 4.5mm. The objective of this study was to evaluate the effect of AZM and PDT on pellet production rate (**PPR**), pellet durability index (**PDI**), and hot pellet temperature (**HPT**) in corn, soybean meal, and dried distillers grains with solubles-based poultry diets, manufactured under a constant motor load. The inclusion of AZM increased overall PPR by 7.9% ($P < 0.001$) and decreased overall PDI by 1.6 percentage points ($P < 0.001$). As PDT increased from 32mm to the average of the 38 and 45mm treatments, PPR decreased by 28.5% ($P < 0.001$) and increased PDI by 10.1 percentage points ($P < 0.001$). A main effect interaction of AZM and PDT was recognized to influence HPT ($P < 0.007$), however, no difference was observed for the 45mm PDT when AZM was included. Linear contrasts showed that PPR increased by 5.0, 7.9, and 11.8% when comparing AZM treatments to their respective controls (All PDT; $P < 0.001$). The authors of this experiment concluded that AZM increased PPR across PDT and decreased PDI, albeit to the extent that would likely not affect broiler performance.

In Chapter 4, a follow-up experiment to the previous was conducted to evaluate the influence of PDT and AZM inclusion rate on broiler performance and apparent ileal amino acid digestibility (**AIAAD**). A 2 (AZM) x 2 (PDT) factorial was utilized in this experiment to create a total of four treatments. The dietary treatments were sourced from the previous AZM experiment, and only the treatments utilizing the 32 and 45mm PDT were utilized. All feed was reground and slightly reformulated to reduce the confounding bias of PDI and to ensure an acceptable nutritional density. Ten randomized complete blocks of raised wire cages were utilized with each pen housing ten Ross 308 broilers. The objective of the study was to determine the effect of pelleted feed using AZM (0.0 or 0.25%) and PDT (32 or 45mm; using a constant die diameter of 4.5mm) on Ross 308 male broiler feed intake, live weight gain, feed conversion ratio, and AIAAD from 0 to 21 days of age. Live performance did not differ due to an interaction or independent main effects ($P > 0.05$). However, AIAAD was influenced by AZM and PDT interactions ($P < 0.05$). The amino acids Ala, Asp, Glu, Gly, Ile, Lys, Met, Pro, Thr, and Val demonstrated increased digestibility with the 45mm Control treatment. This treatment also produced the highest numerical HPT in the feed manufacture experiment. It was hypothesized that the digestibility resulted from increased frictional heat that deactivated trypsin and chymotrypsin inhibitors or degraded ingredient cell wall structure. While both inhibitors were determined to be present using SDS-PAGE and chemiluminescent detection, quantitative analysis showed no practically influential concentrations of either inhibitor or a decrease large enough to explain the increased AIAAD. The authors, therefore, speculate that the increase in AIAAD of various amino acids was due to the breakdown of the aleurone layer cell walls of the corn kernels caused by increased frictional heat exposure vis the 45mm PDT and the absence of AZM.

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ABBREVIATION KEY

I. Chapter One

ADFI –	Average Daily Feed Intake
ADG –	Average Daily Gain
AIAAD –	Apparent Ileal Amino Acid Digestibility
AZM –	Azomite
BW –	Body Weight
CV –	Coefficient of Variation
DCP –	Dicalcium Phosphate
DDGS –	Distillers Dried Grains with Solubles
FCR –	Feed Conversion Ratio
FI –	Feed Intake
GMD –	Geometric Mean Diameter
GSD –	Geometric Standard Deviation
IPS –	Inorganic Phosphate Source
L:D –	Pellet Die Thick to Pellet Diameter Ratio
LWG –	Live Weight Gain
MAF –	Mixer-added Fat
MT/hr –	Metric Ton per Hour
PDI –	Pellet Durability Index
PDT –	Pellet Die Thickness
PPR –	Pellet Production Rate
PQ –	Pellet Quality
TAAD –	Total Amino Acid Digestibility
TCP –	Tricalcium Phosphate

II. Chapter Two

CD –	Complete Diet
Cl^- –	Chloride Ion
CS –	Corn-Salt
CV –	Coefficient of Variation
DM –	Dry Mix
Free Lys –	Crystalline L-Lysine-HCL
Free Met –	Crystalline DL-Methionine

HA – Horizontal + Adequate

HI – Horizontal + Inadequate

MTI – Mix Time

MTY – Mixer Type

MU – Mix Uniformity

TA – Transfer + Adequate

TI – Transfer + Inadequate

VA – Vertical + Adequate

VI – Vertical + Inadequate

WM – Wet Mix

III. Chapter Three

AZM – Azomite

CPM – California Pellet Mill

DDGS – Dried Distillers Grains with Solubles

HPT – Hot Pellet Temperature

L:D – Length to Diameter Ratio

PDI – Pellet Durability Index

PDT – Pellet Die Thickness

PPR – Pellet Production Rate

IV. Chapter Four

AIAAD – Apparent Ileal Amino Acid Digestibility

AZM – Azomite

CIU – Chymotrypsin Inhibitor Units

CTI – Chymotrypsin Inhibitor

FCR – Feed Conversion Ratio

FI – Feed Intake

HPT – Hot Pellet Temperature

LWG – Live Weight Gain

MORT% – Mortality Percentage

PDT – Pellet Die Thickness

TIU – Trypsin Inhibitor Units

TI – Trypsin Inhibitor

CHAPTER ONE

LITERATURE REVIEW

Grinding

Grinding is the process of breaking down grains into smaller, relatively uniform, particles prior to most other steps of feed manufacture (Thomas et al., 2018; Cordova-Noboa et al., 2021). Geometric mean diameter (**GMD**) and geometric standard deviation (**GSD**) are used to quantify the average particle size of ground grain and its relative variability respectively (Nir and Hillel, 1994). In the case of monogastric production animals (poultry and swine), corn is typically the principal ingredient. Therefore, it is of the most concern in terms of grind quality in the feed manufacturing process (Downs et al., 2023). Most, if not all, of the other ingredients utilized in monogastric rations, are already ground or do not require grinding prior to their arrival at the feed mill, and because corn potentially has the largest impact on the average particle size of the feed (Downs et al., 2023). Other grains such as wheat or sorghum are utilized as the primary grain, however, added milling efforts prohibit their cost-effectiveness in many situations in the United States.

Grinding is typically accomplished through two different methods, hammer milling and roller milling, each of which has different mechanisms of grinding. Hammer mills work via impaction of the grain, while roller mills work through compaction and shearing of the grain. Reece and coauthors (1985) found that roller mills produce ground samples of feed with numerically lower GSD (2.23) when compared to a hammer mill (2.35), indicating that roller mills produce a less variable average particle size. The authors of that study also found that the roller consumed less energy compared to the hammer mill (2.42 vs. 2.83 w•hr/kg). Similarly, Thomas and coauthors (2018) found that, on average, roller mills are more energy-efficient than hammer mills (10.02 kJ/kg vs. 23.49 kJ/kg; $P < 0.05$). However, hammer mills tend to be cheaper initially and are easier to operate compared to roller mills (Reece et al., 1985; Hafeez et al., 2015). Hammer mills have also been shown to grind fibrous ingredients more efficiently and consistently than roller mills (Thomas et al., 2018). The authors of the previously mentioned study suggested that the hammer mill was more applicable in grinding fibrous ingredients such as wheat, whereas malleable ingredients such as soybeans were better ground in the roller mill (Thomas et al., 2018).

Grinding efficacy can be influenced by grain type, grain moisture content, grinding method, and desired GMD (Deaton et al., 1995; Thomas et al., 2018; Rubio et al., 2020; Cordova-Noboa et al., 2021; Downs et al., 2023). This is likely due to the variation in grinding mechanisms between the two mills, as hammer mills grind via impaction and roller mills grind via compaction and shear (Thomas et al., 2018). Lyu and coauthors (2022) found that increasing the moisture content of corn from 12.3% to 14.4% increased the average particle size by 0.30 mm when using a hammer mill (1.71 to 2.01mm; $P = 0.043$). The authors of this study also reported increased energy consumption along with increased moisture content, indicating that the grain may have become more resistant to the impaction forces of the hammer mill (Lyu et al., 2022). This means more force was likely required to obtain a particle size small enough to fall through the screen, resulting in larger energy consumption.

Equally important is the potential for grain shrink when grinding higher-moisture grain. When grinding higher-moisture grain in a hammer mill, the grain is exposed to increased friction between the hammer and kernel, thus increasing the temperature of the grain (Lyu et al., 2022). The increased temperature leads to evaporation of the excess moisture, which results in partial loss of the product being ground. Therefore, feed manufacturers are paying for water that can ultimately be lost. There is also the issue of grain spoilage if the moisture content is too high, requiring buyers to dry the grain to ensure storage stability (Sadaka et al., 2017). Probst and coauthors (2013) observed no decrease in moisture content when grinding 10.39% moisture corn in a hammer mill ($P > 0.05$). However, when 16.02% moisture corn was ground, in the same mill, there was a decrease of 1.00% moisture ($P < 0.05$; Probst et al., 2013). Due to this, grain mills, ethanol plants, and co-ops have implemented shrink factors to account for the loss of product when grinding these higher-moisture grains as well as the anticipated losses associated with grain handling during the drying process (Sadaka et al., 2017).

Grain grinding also has been shown to influence the consumption and nutrient utilization of the feed being produced as well as the live weight gain (**LWG**) and feed conversion ratios (**FCR**) of the animals consuming the feed (Nir and Hillel, 1994; Rubio et al., 2020; Downs et al., 2023). Reece and coauthors (1985) also found that male broilers fed mash starter diets ground using a roller mill had larger body weights (2.308 kg) than broilers fed mash starter diets ground using a hammer mill (2,243 kg) at day 47 ($P < 0.05$). This is likely due to the decreased variation in particle size, as the broilers were likely less inclined to sort their feed for larger corn particles (Reece et al., 1985; Deaton et al., 1989). Chewning et al. (2012) found that increasing the particle size of ground corn from 300 to 600 μm increased broiler FCR by 0.31 ($P < 0.001$), increased feed intake (**FI**) by 138 g ($P = 0.001$) and tended to decrease female broiler LWG ($P = 0.052$) from day 0 to 21, indicating that nutrient utilization was not as efficient in the larger particle corn. Conversely, Downs and coauthors (2023) also found that increasing the particle size of ground corn from 832 μm to 1432 μm increased Ca digestibility by 12.2% (44.1 to 56.3%; $P = 0.001$). Therefore, the influence of particle size on nutrient digestibility may be dependent on the ingredient and/or nutrient of concern.

While feed that is ground finer will have more surface area to interact with digestive enzymes and the brush border of the small intestine, certain enzymatic secretions are influenced by digestive organ stimulation. Pacheco and coauthors (2013) hypothesized that the increased FCR seen when feeding fine ground soybean meal is due to the decreased retention and increased passage rate of the feed through the digestive tract. Gabriel et al. (2003) found that broilers fed larger particle feeds, such as whole wheat, had increased gizzard function due to the need for more mastication. The authors also noted that the higher degree of gizzard stimulation may have led to greater H^+ secretion and subsequent pepsin activity, thus leading to increased denaturation and hydrolysis of dietary proteins. Similarly, Nir and Hillel (1994) found that increasing the particle size of the primary grain in a broiler diet from an average of 0.625 to 1.168 mm increased the average broiler empty gizzard weight by 0.58 g/100g of body weight (P = 0.010). The authors of this study attributed the increased gizzard weight to the larger particles causing activation of the gizzard, thus increasing its size relative to the size of the broiler.

Therefore, modifying grain grinding parameters of the primary grain may positively or negatively influence feed manufacturing costs, the digestibility of some nutrients, and the performance of broilers. However, further information about specific situations would be required to appropriately describe these influences. Regardless, the overall goal of grinding grain is to provide a feed that has an acceptable average particle size with minimal variation as this allows for proper processing of the feed later in feed manufacture.

Mixing

The goal of feed mixing is to create a homogenous mixture of different feedstuffs that can be fed to livestock and provide the nutrients required for proper animal maintenance and growth. Particle size, bulk density, fiber content, electrostatic charges of additives, liquid addition, small inclusion-rate ingredients, mixing time, and moisture can all affect the uniformity of feed mixtures (Clark et al., 2007; Adusei-Bonsu et al., 2021). Mix uniformity is typically measured by the coefficient of variation (**CV**), which is the ratio of the standard deviation to the mean, multiplied by 100, and a good mix is considered <10% CV (McCoy et al., 1994; Clark et al., 2007; Groesbeck et al., 2007). The coefficient of variation is found by taking representative and equally spaced samples, typically ten per batch, from mixed feed and measuring the concentration of a selected marker (McCoy et al., 1994; Clark et al., 2007; Groesbeck et al., 2007). The goal of sampling for measuring mix uniformity is to collect enough samples, at the correct locations in the mixer, and at the correct time to get a representative estimation of the mixer's performance. Marker selection also plays a large role in perceived mix uniformity and mixer performance.

Common markers selected for evaluating mix uniformity are chloride ions, dyed iron, free methionine, free lysine, crude protein, phosphorus, manganese, and various medications (McCoy et al., 1994; Clark et al., 2007; Reese et al., 2017). However, each of these markers have advantages and disadvantages regarding practicality, feasibility, and accessibility of inclusion and testing. When selecting a marker, the accuracy of the laboratory assay, ease of the laboratory assay, analysis cost, commonality of the marker, as well as the sources of the marker must be considered to properly evaluate mix uniformity (Axe 1995; Clark et al., 2017). For example, utilizing crude protein as a marker for mix uniformity of a complete diet would not be appropriate as there would be many sources of crude protein in the formulation as well as having a relatively expensive assay cost. However, utilizing chloride ions as a marker for mix uniformity in a corn and salt formulation would be appropriate as the marker is only sourced from one ingredient: salt, and the laboratory assay is relatively cheap and quick.

Limited previous research has shown that as mix uniformity increases, broiler performance increases. Beumer (1991) reported that adequate mix uniformity is one of the most important aspects of feed manufacturing as without it, improper distribution of nutrients would lead to decreased animal performance. According to Ensminger and coauthors (1990), baby chicks only consume a few grams of feed a day, therefore, proper distribution of the essential nutrients is required to ensure proper growth and performance. McCoy et al. (1994) found that when decreasing chloride ion CV in a full ration from 40.5 to 12.1%, day 0 to 21 broiler average daily gain and gain to feed ratios increased from 23.6 to 30.0g and 0.548 to 0.583 respectively ($P < 0.05$). These findings are likely due to more uniform consumption of nutrients in the feed, thus allowing optimal performance.

Mixer type can also influence the amount of time required to obtain an adequately mixed batch of feed. Goodband (1990) writes that horizontal ribbon mixers need 5-10 minutes to achieve <10% CV, while vertical screw mixers need approximately 15 min to reach the same threshold. Similarly, Clark and coauthors (2007) were able to achieve an acceptable mixture in 5 minutes while utilizing a horizontal, double-ribbon mixer. However, Reese and coauthors (2017) required 10 minutes to achieve a similar mix uniformity while utilizing a single-screw vertical mixer. Currently, to our knowledge, there is no published research evaluating the influence of a batch-to-mix transfer system on mix uniformity. Regardless, commercial producers must consider which mixer will work in their operation to best fit their feed manufacturing needs and budget.

The particle size of ground feed, its particle size variation, ingredient density, ingredient addition order, and liquid addition procedures are some of the most influential non-mixer factors on mix uniformity (Fahrenholz 2019). Ingredient density should be accounted for when mixing feeds as denser particles will migrate downward in the mixer, however, this issue may be largely unavoidable in certain circumstances (Axe 1995; Fahrenholz 2019). Ingredient addition order should be thoroughly planned prior to mixing feed as small inclusion ingredients (i.e., minerals, medication, vitamins) may tend to reside in dead zones of the mixers and not be fully incorporated into the mixture (Axe 1995; Fahrenholz 2019). Similarly, adding liquid ingredients, such as oil, at inappropriate times may lead to small inclusion ingredients getting stuck to augers, paddles, ribbons, and/or mixer walls, ultimately leading to poor uniformity. Therefore, an adequate mix time must be provided to allow for the mixing of all the dry ingredients prior to the inclusion of the liquid ingredients (Axe 1994). Lastly, if the GMD of feed is too extreme, or the GSD is too large, mix quality can be negatively affected (Fahrenholz 2019). Therefore, it may be best practice to strive for a reasonable GMD for the specific situation while minimizing GSD to improve mix uniformity.

In conclusion, proper mixing procedures will result in an adequately mixed batch of feed. Mixing is a major quality control point in the process of feed manufacture as it ensures equal distribution of nutrients and ingredients, ultimately allowing uniform and improved growth of any class of farm animal. Therefore, feed manufacturers must be aware of the various factors that influence mix uniformity and should regularly confirm their procedures are producing an adequate mixture. Researchers should continue to investigate the effects of various mixing systems on mix uniformity as well as those from different diet formulations. For example, to our knowledge, no published literature studied the influence of a batch-to-mix transfer system on mix uniformity.

Mash Feed

Mash feed is the typical name for the properly mixed ground ingredients of a feed formulation. Mash feed is typically pelleted or crumbled in the poultry and swine industry (Serrano et al., 2012; Omede and Iji, 2018). When comparing mash and crumbles fed as a starter feed for broilers, crumbles have been shown to increase FI, LWG, body weight (**BW**), and FCR (Cerrate et al., 2009; Serrano et al., 2012; Omede and Iji, 2018; Rubio et al., 2020). Omede and Iji (2018) observed an increase in FI of 32.0 g, an increase in LWG of 61.9 g, and a decrease in FCR of 0.16 when feeding crumbled feed compared to mash feed in broilers from day 0 to 10 ($P < 0.001$). The authors attributed the performance improvement to the increased ease of consumption via the agglomerated feed, as well as increased uniform consumption. Similarly, Serrano and coauthors (2012) found an increase in average daily gain (**ADG**) of 6.6 g, a decrease in average daily feed intake (**ADFI**) of 4.9g, and a decrease in FCR of 0.64 when feeding crumbled feed compared to mash feed to broilers from day 0 to 11. The authors attributed the improvement in performance to decreased feed wastage as well as more efficient consumption of the crumbled feed.

Mash diets have also been shown to increase the weight of the digestive organs such as the gizzard, proventriculus, small intestine, and pancreas relative to other feed forms (Serrano et al., 2013; Omede and Iji, 2018). Omede and Iji (2018) recognized an increase of 0.29, 0.8, 1.4, and 0.12 g per kg of body weight for the liver ($P = 0.042$), gizzard + proventriculus ($P = 0.001$), small intestine ($P = 0.001$), and pancreas ($P = 0.001$) respectively when feeding mash feed compared to crumbles in broilers from day 0 to 10. The authors attributed increased relative organ weights to increased feed retention time within the gizzard and proventriculus, thus promoting higher activities of these digestive organs. Similarly, Chewning and coauthors (2012) saw an average increase in relative gizzard weight of 0.33 g per kg of body weight when feeding mash feed in place of pelleted feed to broilers from day 0 to 44 ($P = 0.039$). The authors of this experiment attributed the increase in relative gizzard weight to increased feed retention time as the feed particles needed to be broken down before moving further in the digestive system.

Mash diets are most commonly fed to laying hens as recent literature has shown no economic benefit in pelleting or crumbling laying hen ration. Ege and coauthors (2019) recognized no difference in egg production rate, egg weight, shell thickness, or content weight percentages when feeding crumbled feed in place of mash from weeks 21 to 52 ($P > 0.05$). However, an FI increase of 7.0 g per day was recognized in the hens fed crumbled feed within the same period. Therefore, the authors recognize that the absence of increased productive performance when crumbling laying hen feed provides enough evidence to feed mash feed directly to reduce the input cost of production. Dijkslag and coauthors (2023) similarly found that laying hens fed crumbles from weeks 30 to 59 had statistically similar egg production rates, egg weights, and shell thicknesses while also maintaining similar BW and BW uniformity percentages compared to laying hens fed mash feed ($P > 0.05$). The authors concluded that pelleting and crumbling feed destined for laying hens may not be economically beneficial. It should also be noted that the high inclusion rate of rock within the die formulations of laying hens (i.e., limestone) may lead to accelerated degradation of pelleting equipment. Therefore, the overall disadvantages of pelleting feed destined for laying hens largely outweigh the marginal performance improvements.

While pelleting and crumbling feed have been shown to have little to no benefit to laying hen performance compared to mash feed, the particle size of the feed may be different. Ege and coauthors (2019) found that hens fed coarse particle feed (1091 μm) had an increased FCR compared to laying hens fed fine particle feed (759 μm) from weeks 21 to 52 ($P = 0.002$). The authors of this study also recognized an increase of 0.18 and 0.03 g per 100g of body weight in the gizzard and pancreas respectively of the laying hens fed larger particle feed compared to fine particle feed. Therefore, GMD and GSD of mash feed may potentially be of greater importance when feeding laying hens as selective consumption and increased organ stimulation could influence production efficiency, ultimately leading to increased prices at the shelf for consumers (Portella et al, 1988; Ege et al., 2019).

In conclusion, mash feed is an important step in the process of obtaining quality pelleted feed. While the performance of broilers, and other monogastric animals destined for slaughter, fed mash feed has been shown to suffer compared to that of feeding pelleted/crumbled feed, laying hens do not follow the same trends. Therefore, producers should be concerned with feeding the correct feed to maximize the production of their type of animal. If mash feed is selected, feed manufacturers likely should focus on the GMD and GSD of the feeds being produced to reduce the amount of selective consumption and unnecessary organ stimulation, and ultimately maximize performance.

Pelleting and Pellet Quality

Pelleting has become the most popular hydro-thermal feed manufacturing technique over the past century since its introduction in the mid-1920s (Coffey et al., 2016; Abdollahi et al., 2018). While pelleting was originally used to increase feedstuff uniformity, palatability, and usefulness, its modern use is focused more on anti-nutrient segregation, improved nutrient digestibility, improved feed prehension, and improved monogastric animal performance (Behnke, 1994; Coffey et al., 2016; Glover et al., 2018; Khalil et al., 2021; Lemons et al., 2021).

Pellet quality (**PQ**) has been of increased concern in literature in recent years, as improvements in PQ have been shown to increase broiler performance (Teixeira Netto et al., 2019; Poholsky et al., 2021; Boltz et al., 2021; Boltz et al., 2023). Pellet durability index (**PDI**) is typically utilized to quantify PQ and is a measure of how well pellets can withstand pressures of conveyance, transport, and feeding. Glover and coauthors (2016) saw that increasing the percentage of pellets from 54 to 69% of the total feed provided decreased FCR by 0.06 when feeding from days 22 to 38 ($P = 0.019$). Lilly and coauthors (2011) found that increasing the percentage of pellets fed from 30 to 90% increased LWG by 39 g from day 21 to 38 ($P < 0.001$). Finally, Corzo and coauthors (2011) saw a decrease in FI of 62 g ($P = 0.01$) and a decrease in FCR of 0.03 ($P < 0.001$) when increasing the percentage of pellets from 32 to 64% in broilers fed to 42 days of age. The authors of these studies attributed the performance improvements associated with increased pellet quality to increased productive energy, decreased nutrient segregation, and increased nutrient utilization. Therefore, it is crucial for producers to feed the highest quality pellet, or crumble for starter feeds, for optimal growth as feed is the largest economic expenditure, at approximately 60-70% of the total cost, when rearing broilers (Lilly et al., 2011; Glover et al., 2016; Rigby et al., 2018, Boltz et al., 2021, Boltz et al., 2023). However, pellet quality can be affected by many factors such as diet formulation, steam conditioning parameters, particle size, and pellet die specifications (Behnke, 1996; Buchanan and Moritz, 2009; Glover et al., 2016).

Traditional poultry formulations are corn and soybean meal-based and also include a liquid fat source and a multitude of micro-ingredients to meet mineral, vitamin, and essential amino acid requirements. Most modern poultry rations also include one or multiple exogenous enzymes to break down anti-nutritional factors such as non-starch polysaccharides and phytate phosphorus (Walters et al., 2018; Gulizia et al., 2022). However, nutritionists and producers are increasingly using non-traditional ingredients to reduce feed costs while maintaining nutritional density (Bottger and Sudekum, 2018; Flores et al., 2019). Corn-derived dried distillers grains (**DDGS**), which is a by-product of ethanol production, is one such non-traditional ingredient that is used largely for its relatively high crude protein content at approximately 24-30% and cheap cost per ton (Fahrenholz, 2005; Swiatkiewicz et al., 2015; Bottger and Sudekum, 2018). Jones and coauthors (2022) found that increasing the DDGS inclusion rate from 0 to 4% increased the average PDI by 5.53 percentage points, however increasing the DDGS inclusion rate from 0 to

16% only increased the average PDI by 10.37 percentage points (P < 0.001). The authors concluded that there may not be a positive linear relationship between the DDGS inclusion rate and PQ. In contrast, Kim and coauthors (2018) found that when increasing the DDGS inclusion rate from 0 to 20%, decreased PDI by 6.4 percentage points (P < 0.01). Therefore, the influence of DDGS on PQ may be largely associated with the corn utilized for ethanol production or the manufacturing process of ethanol.

Another class of ingredients that have been shown to influence PQ is the mixer-added fat (**MAF**) inclusion rate. Fat sources, that are added at the mixer, have been hypothesized and shown to act as a lubricant in the pelleting process as well as provide a protective coating for heat-liable nutrients and enzymes, however, this comes at the cost of PQ (Gehring et al., 2011; Loar et al., 2014; Hossein et al., 2019). Gehring and coauthors (2011) found that PDI decreased by 23.0 percentage points when increased MAF from 1 to 4% (P < 0.001). However, the same increase in MAF % resulted in the sparing of exogenous enzymes (Gehring et al., 2011). Similarly, Loar and coauthors (2014) found that increasing the MAF inclusion rate from 1.0% to 2.2% decreased PDI by 13.0 percentage points (P < 0.001) while decreasing day 28 to 42 FCR by 3 points (P = 0.05). The authors of this experiment attribute the FCR decrease to the increased MAF sparing essential nutrients, which is supported by the 4.6 percentage point apparent ileal amino acid digestibility (**AIAAD**) increase in Thr when increasing MAF from 1 to 2.2% (P = 0.021).

Another factor that has been shown to influence pellet quality is steam conditioning (Behnke, 1996). Steam conditioning is the process of injecting saturated steam into mixed mash, prior to pelleting, to increase the temperature and moisture content of the feed (Boltz et al., 2020). Saturated steam is utilized as the rate of heat transfer is much higher than heated air. The added heat and moisture allow for more reactions between nutrients such as starch gelatinization and protein gelation, which in turn, increases PQ (Cutlip et al., 2008; Buchanan et al., 2010; Boltz et al., 2020). Therefore, increasing the steam conditioning temperature or steam conditioning period could lead to an increased rate of these reactions as more heat and moisture would be added to the feed. Starch gelatinization and protein gelation have been shown to increase the digestibility of their respective nutrients as their enzyme accessibility increases with granule solubilization and denaturation respectively (Thomas et al., 1998; Moritz et al., 2005). Increasing conditioning temperature has also been hypothesized to decrease trypsin inhibitors originating from under-processed soybean meal (Boltz et al., 2020). However, while high conditioning temperatures for extended periods may create high PQ, and potentially deactivate some anti-nutritional factors, it may also lead to essential amino acid degradation, enzyme deactivation, and ultimately, decreased broiler performance (Boney and Moritz, 2017; Lynch et al., 2023).

Boltz and coauthors (2020) showed that increasing the conditioning temperature from 77 to 88° resulted in a PDI increase of 24.8 percentage points (45.7 to 70.5%; $P < 0.001$) and decreased exogenous muramidase enzyme activity by 7441.7 activity units/kg (25,419.4 to 17,977.7 activity units/kg; $P < 0.001$). However, AIAAD of Ala, Asp, Glu, Iso, Leu, Lys, Met, Pro, Thr, and Val were influenced by an interaction of conditioning temperature and conditioning time, with the 88°C + 60-sec treatment consistently resulting in an AIAAD statistically similar to the 77°C + 30-sec treatment ($P < 0.001$). The authors recognized that the longer conditioning time at the highest temperature (88°C) likely deactivated the muramidase enzyme to a large enough degree and conformationally chanced amino acids within the protein, rendering them indigestible to the broilers (Bolts et al., 2020). Similarly, Perera and coauthors (2021) found that increasing the conditioning temperature from 60 to 88°C increased PDI by 4.2 percentage points ($P = 0.021$) while also increasing FCR by approximately 8 points ($P = 0.002$). The authors of this study attributed the decreased broiler performance to increased digesta viscosity and nutrient degradation due to the increased conditioning temperature. Boney and Moritz (2017) also found that increasing conditioning temperature from 74 to 91°C increased PDI by 18.0 percentage points while also decreasing the total amino acid digestibility (**TAAD**) of Ala, Asp, Glu, Leu, Val, and Iso by 2.2-

2.9 percentage points depending on the amino acid ($P < 0.05$) and also tended to decrease the TAAD of Lys ($P = 0.065$). Therefore, the authors attributed the decrease in TAAD observed to the increased conditioning temperature creating conformation changes to amino acids within denatured proteins, rendering them unavailable.

The particle size of the primary ingredient, as well as the complete diet, has been shown to influence PQ. Smaller particle sizes allow for deeper and more thorough penetration of heat and moisture into the feed particles as well as a larger surface area contact between feed particles, thus allowing for increased adhesion during the pelleting process and increased PQ (Wondra et al., 1995; Parsons et al., 2006; Ovi et al., 2021). Chewning and coauthors (2012) noted that as the particle size of ground corn increased from an average of 267 µm to 557 µm the average PDI decreased by 3.3 percentage points. Hossein and coauthors (2019) found that increasing the average GMD of the whole diet from 697 µm to 953 µm decreased PDI by 5.8 percentage points. Finally, Wondra and coauthors (1995) saw that increasing GMD of the whole diet from 517 µm to 1,017 µm decreased PDI by 7.6 percentage points. In each of these studies, the authors attribute the decreased PQ to a smaller degree of agglomeration of feed particles as the surface area to volume ratio decreases as the particle size decreases. However, grinding the primary grain and/or the whole ration to a finer particle size will likely result in an added cost, but, this cost may be offset by the increased performance associated with a higher PQ.

In conclusion, pelleting feed is a complicated procedure that can be influenced by many different factors. Therefore, feed manufacturers should have a clear and thorough understanding of how these factors can affect the quantity and quality of production, what the goal of production is for their operation, and how they can potentially optimize the feed mill they are operating.

Production Rate and Efficiency

With feed and feed manufacture constituting approximately 60-70% of the total cost associated with poultry production, producers are constantly searching to increase efficiency within ration formulation and feed manufacture (McKinney and Behnke, 2007; Cutlip et al., 2008; Pope et al., 2018; Boltz et al., 2021; Bowen et al., 2022). Therefore, increasing the pellet production rate (**PPR**) while maintaining mechanical input costs improves the efficiency of feed manufacture and ultimately results in cost savings for producers and customers. However, PPR can be influenced by many factors such as: diet formulation, conditioning specifications, pellet die specifications, and throughput agents (Behnke, 1996; Buchanan and Moritz, 2009; Glover et al., 2016; Boltz et al., 2021). Generally, factors that influence PQ also influence PPR. Typically, as PPR increases, PQ has been shown to decrease (Behnke, 1994). Traditionally, PPR is quantified and denoted in metric tons per hour (**MT/hr**) or kilograms per minute, however, MT/hr will be used for the remainder of this literature review to compare experiments more effectively.

Previous literature has demonstrated the influence of diet formulation on PPR. Behnke (2007) notes that DDGS have an unusual influence on feed manufacturing as an inclusion rate larger than 5-7% decreased PPR despite the relatively high oil content. The author suggested the likely cause for decreased PPR is due to the relative increase in fiber content, and therefore more friction between the feed and pellet die, as well as soluble proteins adhering to the pellet die chamber (Behnke, 2007). However, Jones and coauthors (2022) found that increasing the inclusion rate of DDGS from 0% to 8% resulted in a PPR increase of 6.5% (0.724 to 0.771 MT/hr; $P < 0.05$). Therefore, similar to its influence on PQ, DDGS may vary in influence on PPR based on the starting material and manufacturing conditions throughout ethanol production.

Another key ingredient in diet formulation is the inorganic phosphate source (**IPS**). Boltz and coauthors (2021) found that utilizing tricalcium phosphate (**TCP**) in place of dicalcium phosphate (**DCP**) increased PPR by 11.8% (0.990 to 1.107 MT/hr; $P < 0.001$) while pellet mill motor load was held constant. Similarly, Bowen and coauthors (2022) found that replacing DCP with TCP to meet the same nutritional density resulted in an increase in PPR of 1.6% (1.23 to 1.25 MT/hr; $P = 0.039$) while pellet mill motor load was held to 42% of its maximum amperage. The authors of both studies attributed the increase in PPR observed to the potential pellet die-scouring abilities of TCP as the particles of this ingredient have a high angularity (Verner, 1988; Wamsley et al., 2012). This pellet die-scouring property is typically associated with the ability of the product to scrape away residual feed that adheres to the interior of the pellet die during the pelleting process. This likely allows for easier passage of feed through the pellet die and ultimately, increases PPR (Behnke, 1981; Boltz et al., 2021; Bowen et al., 2022). It should be noted that the use of IPS in the poultry industry has decreased in recent years due to environmental concerns, increased costs, and the availability and use of exogenous phytase enzymes (Boltz et al., 2021).

Modifying the steam conditioning step associated with pelleting feed has also been shown to influence PPR. Tillman and coauthors (2020) saw an increase in PPR of 11.3% when increasing the conditioning temperature from 82.2 to 87.8°C (0.638 to 0.710 MT/hr; $P < 0.001$). These findings are likely due to the addition of more lubrication via the steam, thus allowing easier passage of feed through the pellet die (Behnke, 1990; Behnke, 1994). Similarly, Loar and coauthors (2014) found that increasing the conditioning temperature from 74 to 85°C decreased pellet mill energy consumption by 15.1% while maintaining a relatively constant PPR (9.883 to 8.386 kWh/t; $P < 0.001$). The authors of this experiment attributed the decreased energy use to the added lubrication from the higher quantity of steam required to achieve the target conditioning temperature. However, the author is unaware of published research concerning the influence of conditioning time on PPR.

While few publications exist, some past literature investigates the influence of varying pellet die specifications on PPR. Pellet die thickness (**PDT**), also called effective thickness of the pellet die, is defined as the length feed must pass through while also being compressed to the desired pellet diameter (Leaver, 1982; Behnke 1994). Traditionally, a ratio between the PDT and pellet diameter (**L:D**) is utilized to relate the effective thickness to the diameter and to better understand the impact on PPR and PQ a pellet die may have. Behnke (1990) found that increasing the PDT from 38.1 to 50.8 mm decreased the PPR of a corn and soybean meal-based poultry diet by 34.6% (0.999 to 0.647 MT/hr; $P < 0.05$). To our knowledge, this is the only currently available research into the influence of PDT on PPR. However, other literature investigated the influence of PDT on PQ and enzyme stability (Truelock et al., 2019; Pope et al., 2020; Smith et al., 2021).

IPS use in poultry rations has steadily decreased in recent years due to the development of exogenous phytase enzymes and concerns about environmental impact. However, reducing, or entirely removing, the IPS fraction from formulations destined for pelleting reduces the overall pellet die-scouring ability, potentially leading to decreased PPR. Therefore, feed manufacturers are investigating and utilizing throughput agents to counteract these negative influences on PPR. Azomite® (**AZM**), has gained popularity and attention in recent years for reports of its positive impact on PPR while maintaining a similar PQ. Azomite is a ground, highly mineralized volcanic ash mined in Utah, USA that is characterized as a *dacitic* (*rhyolitic*) *tuff breccia* (Boltz et al., 2021; Bowen et al., 2022). Azomite is hypothesized to positively influence feed manufacture through two mechanisms. The coarse fraction of AZM, which has an angular shape similar to that of TCP, has been hypothesized to scour the pellet die chamber, allowing easier passage of feed through said pellet die chamber, and ultimately increasing PPR (Boltz et al., 2021; Boltz et al., 2023). Similarly, the fine fraction of AZM is hypothesized to interact with water to create a lubricating film within the pellet die chamber, thus increasing PPR due to less friction between the feed and pellet die (Boltz et al., 2021; Boltz et al., 2023).

Tillman and coauthors (2020) reported a PPR increase of 3.3% (0.657 to 0.679 MT/hr; $P < 0.001$) when incorporating AZM at 0.25% of the whole diet, while also maintaining a similar PQ ($P = 0.855$). Boltz and coauthors (2021) found an interaction between IPS and AZM, with the formulations containing TCP and 0.25% AZM resulting in the highest PPR ($P = 0.014$). The authors cite the angular shape of TCP and AZM working to scour the pellet die chamber of residual feed, thus allowing easier throughput of feed, and increasing PPR. Boltz and coauthors (2021) also proposed that the fine fraction of AZM, in combination with water, acted as a lubricant within the pellet die camber; also working to increase PPR within their experiment. Bowen and coauthors (2022) found that including AZM at 0.25% of the diet decreased the pellet mill motor load by 0.5% of its maximum amperage while maintaining a constant PPR of 1.24 MT/hr ($P < 0.001$). The authors also found that IPS and AZM interacted to affect the AIAAD of Cys, with the TCP w/ 0.0% AZM and DCP w/ 0.25% AZM treatments providing the highest values of 77.9 and 77.6% respectively, whereas the DCP w/ 0.0% AZM had the lowest AIAAD of Cys at 75.5% ($P < 0.05$). Therefore, Bowen and coauthors (2022) noted that the increased AIAAD of the heat-liable amino acid Cys in diets manufactured with TCP or AZM was likely due to less friction between the feed and pellet die due to die-scouring abilities of these products. Therefore, there was a reduction in the magnitude of conformational changes to the protein content of the feed, ultimately increasing the AIAAD. In a recently published study, Boltz and coauthors (2023) found that when used independently, the coarse and fine fractions of AZM provided PPR values similar to that of a formulation devoid of any AZM ($P > 0.05$). However, including AZM in its desegregated form, at 0.25% of the diet, increased PPR by 6.8% compared to the control formulation (0.935 to 0.999 MT/hr; $P = 0.004$) while also maintaining a similar PQ ($P = 0.173$; Boltz et al., 2023). Therefore, AZM may be a beneficial ingredient to increase PPR, feed manufacturing efficiency, and AIAAD while also maintaining a similar PQ.

Conclusion and Applications

An efficient and effective feed mill manager or poultry producer must have a clear and thorough understanding of feed manufacture to best meet the needs and goals of their company, regardless of the complexity of the topic. Feed manufacturing is constantly changing and adapting to genetic advancements, nutrient requirements, disease threats, as well as consumer perceptions. Therefore, research is required to understand how these changes influence productivity, feed quality, and profitability.

Future areas of research could potentially encompass:

- The effect of mix uniformity on large animal performance (calves, foals, lambs)
- The effect of grinding mill and particle size on mix uniformity, pellet quality, pellet production rate, energy consumption, and broiler performance.
- The effect of pellet diameter and Azomite on pellet quality, pellet production rate, and broiler performance.
- The effect of micro pellets compared to crumbles fed to broilers within the starter phase of production.

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Chapter Two

A Horizontal Mixer and a Batch-to-Horizontal Mixer System Increased the Mix Uniformity of Free Methionine in Complete Diets Relative to a Vertical Mixer

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SUMMARY

Mix uniformity (MU) has been shown to be affected by mixer type (MTY) and mix time (MTI). The coefficient of variation (CV) of a selected nutrient/ingredient marker is typically utilized to measure MU. An industry-acceptable CV is < 10%, which is calculated based on the analysis of ten representative samples taken from a batch of mixed feed. The objective of this experiment was to determine the effect of a vertical mixer, horizontal mixer, and batch-to-mix transfer system on mix uniformity while utilizing inadequate or adequate mixing times. Chloride ions (Cl^-), crystalline DL-Methionine (Free Met), and crystalline L-Lysine-HCl (Free Lys) were used as markers to calculate the CV. Feed was mixed in a 2 (MTI) x 3 (MTY) factorial arrangement that was replicated three times. Increasing MTI decreased Cl^- CV by 3.91% ($P = 0.017$). An interaction between MTY and MTI affected Free Met CV ($P = 0.005$). The horizontal mixer and transfer mixing system did not demonstrate changes in the Free Met CV compared to the vertical mixer when MTI was manipulated. The authors hypothesize that additional mixing during load-out and added conveyance caused this interaction. The transfer mixing system resulted in the lowest Free Lys CV ($P = 0.017$); however, no treatment provided an industry-acceptable MU. In conclusion, Free Lys may not be an appropriate marker for MU, Free Met may be an appropriate marker for MU in complete diets, and the transfer mixing system may contribute to an acceptable MU.

Keywords: Mix Uniformity, Coefficient of Variation, Mixing

DESCRIPTION OF PROBLEM

Feed and feed manufacture constitute approximately 60-70% of the total poultry production cost, thus a large proportion of economic profit can be attributed to the quality of the feed being produced (McKinney and Behnke, 2007; Boltz et al., 2021; Knarr et al., 2023). One of the major quality control points of feed manufacture, especially for animals with low feed intake (chicks, piglets, and poults), is to create a pellet that discourages selective feeding and provides an even distribution of all ingredients and nutrients. Proper mix uniformity (**MU**) initiates the production of pellets that will support optimal health and performance. Mix uniformity is commonly measured via the coefficient of variation (**CV**), which is the ratio of the standard deviation to the mean of a set of selected samples. Typically, 10 samples are collected from a batch of feed to be analyzed for a particular nutrient or ingredient. Those nutrient/ingredient marker concentrations are then utilized to calculate the CV, with a proper mix uniformity designated as a CV of less than 10% (McCoy et al., 1994; American 2005; Clark et al., 2007; Groesbeck et al., 2007). McCoy and coauthors (1994) found that decreasing the chloride ion (**Cl^-**) CV by approximately 29% (40.5 to 12.1%; $P < 0.05$), via a longer mixing time, increased average daily gain and gain:feed by 6.5 g and 0.035 respectively in broilers from 0 to 21 days of age ($P < 0.05$). However, MU can be affected by many factors such as particle size, bulk density, mixer type (**MTY**), mix time (**MTI**), mixer over-filling, ingredient addition order, ingredient inclusion rate, sample collection location, sample frequency, and CV marker selection (Goodband 1990; Clark et al., 2007; Reese et al., 2017; Fahrenholz 2019; Adusei-Bonsu et al., 2021).

Clark and coauthors (2007) found that the CV of crystalline DL-Met (**Free Met**) decreased by approximately 14.4% and the CV of crystalline L-Lys-HCL (**Free Lys**) decreased by approximately 11.1% in a horizontal, double-ribbon mixer when increasing the mixing time from a 0-second dry mix (**DM**) and a 30-second wet mix (**WM**) to a 120-second DM and a 180-second WM, in a complete diet ($P < 0.05$). Similarly, Reese and coauthors (2017) found that Cl^- CV decreased by approximately 14.0% in a vertical, single-screw mixer when increasing the DM time from 30 seconds to 10 minutes, in a corn-salt ration ($P = 0.003$). The authors are not aware of published research on the effect of a batch-to-mix transfer system on MU. A transfer system involves the conveyance of batched feed to a mixer to be mixed.

Marker selection has also been shown to play a role in perceived MU. If an inappropriate marker is selected to measure MU, artificially inflated or deflated CV values may result, thus potentially leading to improper corrective action and decreased broiler performance. Therefore, the marker selected must only come from one source in the diet, be included in a high enough concentration to measure, and have a relatively cheap analysis (Clark et al., 2007; Rocha et al., 2015; Reese et al., 2017). One common marker used to evaluate MU is Cl^- derived from salt, which is a cheap and commonly used ingredient in diet formulations (American, 2005; Clark et al., 2017). The lab analysis for Cl^- is also relatively cheap compared to other markers and can be done on-site (American, 2005; Hach, 2023). However, using Cl^- as a marker for MU requires a mixture where there is only one source of Cl^-, for example, a mixture of corn and salt. Therefore, a mixture of corn and salt may not be a time-effective evaluator of MU in the busy schedule of a commercial or integrated feed mill.

To evaluate MU while utilizing a mixture that would be applicable in industry use, crystalline Met and crystalline Lys may be used (Behnke 1996; Clark et al., 2007; Rocha et al., 2015). However, laboratory analyses to test for these markers must only be for supplemental Met and supplemental Lys to ensure that only the crystalline fractions of these amino acids are being reported, thus providing a single source marker. While these analyses tend to cost more per sample, the ability to test feed destined for commercial feeding may make MU testing more feasible and representative for industry feed mill use as well as integrating better into daily production goals. The selection of these markers also allows producers to incorporate an oil fraction in the experimental mixture, thus allowing testing of a DM and WM for a single batch of feed.

Therefore, the objective of this experiment was to determine the effect of three different mixing systems: a vertical screw mixer, horizontal double ribbon, paddle mixer, and a batch-to-mix transfer system on mix uniformity while utilizing inadequate or adequate mixing times. The coefficient of variation was calculated using three different markers: Cl^-, Free Met, and Free Lys.

MATERIALS AND METHODS

Factors and Treatments

In this experiment, formulations of a corn-salt (**CS**) mixture or a complete diet (**CD**) were manufactured in a 2 (MTI: adequate and inadequate) x 3 (MTY: vertical, horizontal, and transfer) factorial arrangement of treatments. The treatments were as follows: Vertical + Adequate (**VA**), Vertical + Inadequate (**VI**), Horizontal + Adequate (**HA**), Horizontal + Inadequate (**HI**), Transfer + Adequate (**TA**), and Transfer + Inadequate (**TI**). Three replications of manufacture were completed for each ration in this experiment. The authors define the adequate mixing time as a 4-minute DM followed by a 4-minute WM for the treatments utilizing the vertical mixer, and a 2-minute DM followed by a 2-minute WM for the treatments utilizing the horizontal mixer. The inadequate mixing times are defined as a 0-second DM and a 0-second WM for both MTY. The TA and TI treatments utilized the horizontal adequate and inadequate mixing times as the traditional mixing took place in the horizontal mixer prior to sample collection. Both the adequate and inadequate mixing times, of both mixers, were selected based on the mixer manufacturer's recommendations (EAI, 2023; Scott, 2023).

A one-ton, Easy Automation Inc. Modular Feed Processor vertical screw mixer was utilized for the VA and VI treatments. Once mixing was completed for the VA and VI treatments, the feed was subjected to approximately 9.9 meters of vertical conveyance using a screw auger followed by approximately 9.1 meters of deadfall into a sack-off bin. A 0.5-ton, Scott Equipment twin-shaft horizontal double paddle, ribbon mixer was utilized for the HA and HI treatments. Once mixing was completed for the HA and HI treatments, the mixed feed was conveyed approximately

5.8 meters with a paddle drag, followed by approximately 9.9 meters of vertical screw auger conveyance, and finally approximately 9.1 meters of deadfall into the sack-off bin. Feed ingredients for these four treatments were added to their respective mixers via the top access hatches while the equipment was not in operation. Once all of the DM ingredients were added, the mixer was engaged for the appropriate MTI and left running during the load out of feed for approximately 35 seconds.

In the treatments evaluating the effect of a feed transfer from a batching location to a mixing location, the ingredients were first loaded into the vertical mixer through the top access hatch and conveyed to the horizontal mixer for traditional mixing. All DM ingredients were added, in their respective order, for each ration, while the vertical mixer was not operating. Once all of the DM ingredients were added, the vertical screw mixer was engaged. The batched feed then left the vertical mixer to be exposed to approximately 9.9 meters of vertical conveyance using a screw auger followed by approximately 7.0 meters of deadfall, and finally, approximately 6.1 meters of horizontal conveyance using a paddle drag prior to entering the horizontal mixer. The horizontal mixer was not engaged until all of the feed being transferred reached the mixer, however, the vertical screw mixer was engaged during the entire 35-second load-out process. This design simulates industry practices of separate batching and mixing systems and was used to test the influence of the necessary conveyance on MU.

Corn-Salt Ration

The CS mixture (Table 1) was formulated based on methodologies of past research that utilized Quantab® chloride ion test strips, and 1% utilized NaCl (American, 2005; Reese et al., 2017). Each treatment was manufactured in triplicate within randomized complete blocks, and only the DM times previously outlined were used as no liquid ingredients were used in this ration. For each batch of feed mixed, the corn fraction was added first through access hatches located on the tops of both the horizontal and vertical mixers, followed by the salt fraction. Feed was added in this manner to avoid mixing while loading was completed, ultimately reducing confounding bias. This also reduced the likelihood of the marker residing within the dead zones of the mixers. Once all of the ingredients were added to the appropriate mixer, that mixer was turned on for the allotted amount of time, and conveyance to the sack-off bin took place while the machine remained engaged.

Complete Diet

Similarly, to the CS mixture, the CD (Table 1) was formulated based on past research and the use of the common laboratory analyses of Free Met and Free Lys (Clark et al., 2007; Reese et al., 2017). Treatments were manufactured in a triplicate of randomized complete blocks. However, both the DM and WM times were utilized as soybean oil was formulated in the CD. For each batch of feed, the corn was added first, then the soybean meal, then the meat and bone meal, then the dried distiller's grains with solubles, and finally the premix of the remaining ingredients. For the treatments to receive adequate mixing time, the dry ingredients were mixed for the respective times and oil was incorporated via the top access hatch located at the center of the mixers after the DM segment. The feed was then allowed to mix for the remainder of the WM segment and conveyed out of the mixer to the sack-off bin following the conclusion of the WM while the mixer was engaged. The treatments receiving the inadequate mixing time were engaged for approximately 2-3 seconds and then the oil was incorporated. Conveyance of mixed feed to the sack-off bin occurred while the allotted mixer was still engaged for approximately 35 seconds.

Sample Collection and Marker Analyses

Bagging of mixed feed took place in a method that avoided the build-up of feed in the sack-off bin, thus reducing the possibility of ingredient segregation and further mixing at this location. Once all the feed had been allocated into approximately 12, 22.68 kg bags, one 500 g sample of the feed was collected from 10 bags for each treatment, excluding the first and last bag. Sample collection for both the CS mixture and CD was performed using the same methodology. This procedure ensured that the sample being collected was not contaminated with residual feed in the auger and conveyor systems and avoided the collection of the fine particles that tend to congregate in the final bag of each batch.

Following feed manufacture, each sample from the CS ration was subjected to Cl^- concentration analysis utilizing 300-6000 ppm chloride ion Quantab® titrator strips. This procedure is a modified version of the protocol outlined in Feed Manufacturing Technology V (2005) and was designed to reduce analytical error. One intermediate sample from two high-uniformity batches of feed was selected and 10 representative sub-samples of each were subjected to Cl^- analysis. These data were then utilized to calculate the CV of the Cl^- assay in duplicate. authors, therefore, recognized that this assay has an average CV of 8.30%. The Cl^--testing procedure is as follows:

1. Fill a 2 L beaker with approximately 1.5 L of deionized water and bring its temperature to 60°C utilizing a Fisher Scientific: Isotemp® hot plate.
2. Weigh 10g of each feed sample from the batch into a 10 mL weigh boat.
3. Fill a graduated cylinder with 100 mL of DI water, pour it into a 150 mL Erlenmeyer flask, and add the 10 g of feed sample to the flask.
4. Utilizing a Thermolyne: Big Bill SE rotator/shaker table, mix the contents for 30 seconds, let rest for 60 seconds, and finally mix for another 30 seconds at approximately 300 rpm.
5. Utilizing a number 41 Whatman filter paper, filter the solution into a 250 mL beaker: allowing for complete filtration.
6. Once all sample filtrations have been completed, place one 300-6000 ppm chloride ion Quantab titrator strip into the solution and allow for the yellow marker to turn blue.
7. Read and record the measurement to the nearest 0.5 mark.

Following feed manufacture, each sample of the CD was split into two representative samples, and one of each sample, from each batch of feed, was analyzed for Free Met and Free Lys at the University of Missouri Feed Labs (Experimental Station Chemical Laboratories, University of Missouri-Columbia, Columbia, MO). The samples were analyzed using the AOAC Official Method 999.13, which solely reported the concentration of crystalline methionine and crystalline lysine within each sample, rather than the total concentration of these amino acids (AOAC, 1982). The Cl^-, Free Met, and Free Lys concentrations were then utilized to calculate the CV of each treatment, in each replication utilizing the following equation.

$$CV\ \% = \left(\frac{\sqrt{\frac{\sum (X_i - \mu)^2}{N-1}}}{\mu} \right) \times 100$$

Where…

CV = Coefficient of variation

X_i = Sample "i" concentration of chloride ion

μ = The mean of samples X_i

N = The number of samples per batch of feed (10 samples for every calculation)

Particle Size

The particle size of each ration, as well as the ground corn, soybean meal, salt, DL-Methionine, and L-Lysine, were measured utilizing a WX Tyler Ro-Tap RX-29 Sieve Shaker. This was done to describe the particle size and provide data for speculations of the potential influence of the varying particle sizes on MU. For each measurement, 100g of representative sample was collected and placed in the Ro-Tap at the top of the stack of sieves. Each sample was then subjected to 10 minutes of pressure in the enclosed sieves. Sieve sizes, from top to bottom, are as follows: 4.75 mm, 3.35 mm, 2.36 mm, 1.70 mm, 1.18 mm, 850 µm, 600 µm, 425 µm, 300 µm, 212 µm, 150 µm, 106 µm, and 75 µm. Once finished, each sieve was removed, weighed, emptied, and reweighed to measure the amount of sample retained by each sieve. These weights were then used to calculate the geometric mean diameter (average particle size) and the standard deviation for each sample. Each mixture and ingredient was analyzed in duplicate.

Statistical Analysis

Data from this study were analyzed in a 2 (MTI) x 3 (MTY) factorial arrangement of treatments that were replicated three times in a randomized complete block. The experimental unit was one 272 kg batch of feed. The factorial analysis was performed to compare the main effect means, as well as determine the interactions of MTI and MTY for the three different mixer uniformity markers. A Log_{10} transformation was performed on the CV data for the Free Lys marker to stabilize the variances. However, no data transformation was done for the Cl^- and Free Met marker CV data. Data were then analyzed in a two-way ANOVA model utilizing JMP Pro 16.2 (SAS Institute, 2021), with the significance level set at $P < 0.05$, and the trend level set at $P < 0.10$. Tukey's HSD was then used, post hoc, to examine multiple treatment comparisons and level effects within each factor if significance was observed for the interaction or main effect. Contrasts, selected based on system comparisons, were also analyzed.

RESULTS AND DISCUSSION

Particle Size

Average particle size, and their respective standard deviations, for each mixture, main ingredient, and marker are shown in Table 2. The authors feel that the influences of particle size on MU in this study may be due to the particle sizes of the markers themselves, as well as the other minor ingredients, as the same corn was utilized throughout both mixtures. While past research indicates that particle size can have a large influence on MU, the authors recognize that the particle sizes of the markers utilized in this experiment were largely fixed (Axe 1995; Behnke 1996; Clark et al., 2007; Fahrenholz 2019).

Corn-Salt Mixture: Cl^- Marker

No interaction between MTY and MTI on Cl^- CV was observed (Table 3). However, increasing MTI decreased Cl^- CV by 3.91% (13.556 to 9.647%; P = 0.017; Table 3). These data support the experimental design, confirming that selected mix times, on average, were appropriate to demonstrate a non-uniform mix with the Cl^- in a CS mixture. Similarly, Reese and coauthors (2017) found that increasing mixing time from 30 seconds to 10 minutes, for a corn-salt ration, decreased Cl^- CV by 13.95% in a vertical screw mixer (21.61 to 7.66%; P = 0.003). In contrast, Clark and coauthors (2007) recognized no significant difference in Cl^- CV when increasing MTI from 30 seconds to 5 minutes in a horizontal mixer (P > 0.05). Rocha and coauthors (2015) also saw no numerical difference in Cl^- CV when increasing the MTI from 50 seconds to 155 seconds in a horizontal mixer (3.19 to 3.19%; P > 0.05). However, both studies utilized complete diets that had multiple Cl^- sources (Salt, L-Lys-HCL, Choline Chloride). Therefore, when comparing the results of the current experiment to the previous studies, it can be reasoned that Cl^- is only applicable as a MU marker when there is only one source (i.e., CS rations; American, 2005). The vertical screw mixer tended to have the highest average CV (14.149%) when utilizing the Cl^- marker, while the transfer system tended to have the lowest (9.418%; P = 0.054; Table 3).

Key contrasts of certain treatments were completed to examine the influence of the transfer mixing system on MU (Table 4). Contrasts of the VA vs. VI, HA vs. HI, and TA vs. TI showed an Inadequate to Adequate decrease of 7.10% (17.701 to 10.597%; P = 0.010), 4.18% (13.327 to 9.147%; P = 0.099), and 0.44% (9.639 to 9.198%; P = 0.853) respectively. Therefore, the Vertical mixer was able to achieve an industry-acceptable MU while utilizing the manufacturer's recommended mixing times (EAI, 2023), while the horizontal mixer trended toward the same result (Scott, 2023). However, the batch-to-mix transfer system showed no statistical or practical difference in Cl^- CV when increasing MTI. For this reason, the transfer system may be able to decrease the overall mixing time while also allowing producers to batch the next mix at the same time, ultimately increasing productivity in the feed mill while maintaining an acceptable MU. This is further supported by the contrasts of VA vs. TA & TI and HA vs. TA & TI providing no significant or practical difference in Cl^- CV (P = 0.571, 0.896). Regardless of the tested mixing times, the transfer mixing system may be able to produce feed as uniform as the Vertical and Horizontal mixers using adequate mixing times. This was likely due to added mixing from the 35-second conveyance and unloading processes associated with the transfer mixing system.

Complete Diet: Free Met Marker

An interaction between MTY and MTI on Free Met CV was observed (Table 5). The vertical mixer demonstrated an increased CV when utilizing the inadequate MTI ($P < 0.05$). However, the CV was not increased within other MTYs, regardless of MTI ($P > 0.05$). This may be due to additional mixing occurring while the horizontal mixer was emptying, as well as mixing occurring within the conveyance, ultimately counteracting the influence of decreased mixing times. Therefore, producers may be able to decrease the amount of time feed is exposed to manufacturer-suggested mixing as some may occur within loadout as well as during conveyance to the next step of feed manufacture, which can increase the efficiency of production.

Key contrasts of interest were performed to examine the influence of the transfer mixing system on MU (Table 6). Contrasts of the VA vs. VI treatments showed an Inadequate to Adequate Free Met CV decrease of 19.00% (27.29 to 8.30%; P = 0.001). Similarly, Reese and coauthors (2017) found that increasing the total mixing time of a complete diet in a vertical screw mixer from 30 seconds to 10 minutes resulted in an analyzed Free Met concentration that was more representative of the calculated value (P = 0.020). These data once again support the manufacturer's recommendations on MTI (EAI, 2023). Contrasts of the HA vs. HI and TA vs. the TI treatments showed an Inadequate to Adequate numerical Free Met CV increase of 1.24% (8.11 to 9.24%; P = 0.619) and 2.76% (8.40 to 11.16%; P = 0.326), respectively. However, the results of the HA vs. HI treatment contrast do not reflect past research nor support the manufacturer’s recommendations (Scott, 2023). Clark and coauthors (2017) found that increasing total mix time from 30 seconds to 5 minutes in a horizontal, double ribbon mixer decreased Free Met CV by 14.39% (23.86 to 9.47%; $P < 0.05$). Based on this discrepancy, the authors of the current study believe that the unloading mechanism from the horizontal mixer to the sack-off bin may have allowed a substantial amount of traditional mixing for the CD when utilizing a horizontal mixer. A similar explanation may also be appropriate for the TA vs. TI contrast. However, the increased difference between these treatments, compared to the HA vs. HI contrast, may be a result of a larger degree of over-mixing as there was an increased conveyance distance for these treatments.

Contrasts of the Free Met data of the VA vs. TA & TI and HA vs. TA & TI treatments show that the combination of the TA and TI treatments are not statistically or practically different from the VA ($P = 0.532$) or HA ($P = 0.883$) treatments (Table 6). Therefore, the authors believe that the horizontal mixer, and the associated systems, may be able to produce adequately mixed complete feed in a shorter time frame compared to the vertical mixer, and in a shorter time than the manufacturer's recommendations. Additionally, a similar conclusion can be made for the transfer mixing system when analyzing the Free Met marker data, as was for the Cl^- marker data, in this system may increase feed mill efficiency. Hence, batching of the next batch can occur at the same time as mixing of the current batch, all while potentially maintaining an acceptable MU.

Complete Diet: Free Lys Marker

An interaction trend was observed for MTY and MTI on Free Lys CV in the FF ration ($P = 0.099$; Table 5). Based on this trend, the VA treatment had the highest CV (31.971%), and the TA treatment had the lowest (19.237%; $P < 0.05$). The VA, HA, HI, and TI treatments did not differ from any other treatment ($P > 0.05$). However, the Free Lys CV averages for every treatment were well above the aforementioned industry target of < 10% and did not follow similar trends to that of the Free Met marker (American, 2005; Clark et al., 2007). Similarly, Reese and coauthors (2017) found that when increasing the mixing time in a vertical screw mixer from 30 seconds to

10 minutes, the Free Lys concentration of the analyzed feed remained similar to the calculated value (P = 0.552). Therefore, based on the current experiment, as well as the Reese and coauthors 2017 experiment, the authors believe that Free Lys may not be an appropriate marker for MU in a CD.

CONCLUSIONS AND APPLICATIONS

1. The horizontal mixer and transfer mixing system did not demonstrate changes in the Free Methionine coefficient of variation compared to the vertical mixer when mix time was manipulated.
2. Chloride ions and Free Methionine may be appropriate markers for assessing mix uniformity in a corn-salt mixture and complete diets, respectively.

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TABLES AND FIGURES

Table 1. Mixture formulations, calculated nutrient content, and CV marker content of the complete diet and corn-salt mixture

Ingredient	Percentage	
	Complete Diet	Corn-Salt Mixture
Corn	65.05	99.00
Soybean Meal (46%)	18.79	-
Meat and Bone Meal	5.00	-
DDGS	5.00	-
Soybean Oil	3.22	-
Dicalcium Phosphate	1.24	-
Limestone	0.68	-
Salt	0.28	1.00
Vit/Min Premix[1]	0.25	-
DL-Methionine	0.21	-
L-Lysine	0.19	-
L-Threonine	0.05	-
Sodium Bicarbonate	0.04	-
Calculated Nutrient %		
ME (Kcal/kg)	1421	1469
C. Protein	17.97	7.64
Ca	1.06	0.05
AvP	0.49	0.09
Cl	0.20	0.60
Methionine	0.46	0.13
TSAA	0.68	0.28
Lysine	0.90	0.21
Arginine	0.93	0.36
Threonine	0.57	0.22
Valine	0.64	0.31
Calculated Free A.A. and Cl, %		
Methionine[2]	0.206	-
Lysine[3]	0.148	-
Chlorine[4]	0.153	0.50

[1] 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.

[2] Calculated using the crystalline Methionine inclusion rate multiplied by its Methionine content.

[3] Calculated using the crystalline Lysine inclusion rate multiplied by its Lysine content.

[4] Calculated using the salt inclusion rate multiplied by its chloride ion content.

Table 2. Averages and respective standard deviations of particle size based on mixture or ingredient

Ration/Ingredient	Average Particle Size	SD of Particle Size
Corn-Salt Ration	1025.359	2.294
Full Feed Ration	986.782	2.168
Ground Corn	1049.578	2.238
Soybean Meal	986.020	1.797
Salt	785.685	1.352
DL-Methionine	247.604	1.733
L-Lysine	547.070	1.820

Table 3. Mixer coefficient of variation based on mixer type and mixing time in the corn-salt mixture

Manufacture Treatment	Corn/Salt CV[1]
Vertical w/ Adequate	10.597
Vertical w/ Inadequate	17.701
Horizontal w/ Adequate	9.147
Horizontal w/ Inadequate	13.327
Transfer w/ Adequate	9.198
Transfer w/ Inadequate	9.639
Treatment SEM[2]	2.863
Treatment P-Value	0.020
Mixer Type	
Vertical	14.149
Horizontal	11.237
Transfer	9.418
Mixer Type SEM	1.253
Mixer Type P-Value	0.054
Mix Time	
Adequate	9.647[b]
Inadequate	13.556[a]
Time SEM	1.023
Time P-Value	0.017
Interaction SEM	1.653
Interaction P-Value	0.173

a-b Means within a column not sharing a common superscript differ significantly ($P < 0.05$)

1 Coefficient of Variation

2 Standard Error of the Mean

3 Least Significant Difference Value

Table 4. Specific contrasts of interest and statistics for the Cl^- marker in the corn-salt mixture

	Cl^- CV %
Vertical w/ Adequate vs. Vertical w/ Inadequate	
Adequate	10.597^b
Inadequate	17.701^a
SEM[2]	2.338
P-Value	0.010
Horizontal w/ Adequate vs. Horizontal w/ Inadequate	
Adequate	9.147
Inadequate	13.327
SEM	2.338
P-Value	0.099
Transfer w/ Adequate vs. Transfer w/ Inadequate	
Adequate	9.198
Inadequate	9.639
SEM	2.338
P-Value	0.853
Vertical w/ Adequate vs. Transfer w/ Adequate and Transfer w/ Inadequate	
Vertical w/ Adequate	10.597
Combination[3]	9.403
SEM	2.024
P-Value	0.571
Horizontal w/ Adequate vs. Transfer w/ Adequate and Transfer w/ Inadequate	
Horizontal w/ Adequate	9.147
Combination	9.403
SEM	2.024
P-Value	0.896

a-b Means within a column not sharing a common superscript differ significantly ($P < 0.05$)

1 Comparisons were selected based on practical applications and subsequent questions that may arise

2 Standard Error of the Mean

3 The value represented is the average of the means for the Transfer w/ Adequate and Transfer w/ Inadequate treatments

Table 5. Mixer coefficient of variation based on mixer type and mixing time in the complete diet

Manufacture Treatment	Met. CV	Lys. CV
Vertical w/ Adequate	8.295^{b}	25.142
Vertical w/ Inadequate	27.294^{a}	31.971
Horizontal w/ Adequate	9.340^{b}	25.468
Horizontal w/ Inadequate	8.105^{b}	20.218
Transfer w/ Adequate	11.161^{ab}	19.237
Transfer w/ Inadequate	8.400^{b}	19.946
Treatment SEM[2]	1.405	4.407
Treatment P-Value	0.007	0.029
Mixer Type		
Vertical	15.046^{a}	28.557^{a}
Horizontal	8.701^{b}	22.843^{ab}
Transfer	9.682^{ab}	19.592^{b}
Mixer Type SEM	1.149	1.958
Mixer Type P-Value	0.038	0.017
Mix Time		
Adequate	9.527	23.283
Inadequate	12.294	24.045
Time SEM	1.120	1.469
Time P-Value	0.138	0.720
Interaction SEM	1.217	2.544
Interaction P-Value	0.005	0.099

a-b Means within a column not sharing a common superscript differ significantly ($P < 0.05$)

1 Coefficient of Variation

2 Standard Error of the Mean

3 Least Significant Difference Value

Table 6. Specific contrasts of interest and statistics for the Free Met and Free Lys markers in the complete diet

	Free Met. CV %	Free Lys. CV %
Vertical w/ Adequate vs. Vertical w/ Inadequate		
Adequate	8.295^{b}	25.142
Inadequate	27.294^{a}	31.971
SEM[2]	1.320	3.598
P-Value	0.001	0.082
Horizontal w/ Adequate vs. Horizontal w/ Inadequate		
Adequate	9.340	25.047
Inadequate	8.105	20.218
SEM	1.320	3.598
P-Value	0.619	0.170
Transfer w/ Adequate vs. Transfer w/ Inadequate		
Adequate	11.161	19.237
Inadequate	8.400	19.946
SEM	1.320	3.598
P-Value	0.326	0.847
Vertical w/ Adequate vs. Transfer w/ Adequate and Transfer w/ Inadequate		
Vertical w/ Adequate	8.295	25.142
Combination[3]	9.682	19.592
SEM	1.272	3.116
P-Value	0.532	0.100
Horizontal w/ Adequate vs. Transfer w/ Adequate and Transfer w/ Inadequate		
Horizontal w/ Adequate	9.340	25.468
Combination	9.682	19.5915
SEM	1.272	3.116
P-Value	0.883	0.084

a-b Means within a column not sharing a common superscript differ significantly ($P < 0.05$)

1 Comparisons were selected based on practical applications and subsequent questions that may arise

2 Standard Error of the Mean

3 The value represented is the average of the means for the Transfer w/ Adequate and Transfer w/ Inadequate treatments

Chapter Three

Azomite®, a *Dacitic (Rhyolitic) Tuff Breccia*, included at 0.25% in Feed Manufactured with 32, 38, and 45mm Pellet Die Thicknesses Increased Pellet Production Rate by 5.0, 7.9, and 11.8%, Respectively.

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Primary Audience: Feed Manufacturers, Researchers, Nutritionists

SUMMARY

Azomite (AZM) has been shown to increase pellet production rate (PPR) when included in corn and soybean meal-based diets containing dicalcium phosphate and dried distillers grains with solubles. It is presumed that these increases in PPR are due to the scouring and lubrication properties associated with AZM. The objective of this study was to determine the effect of AZM (0.25% diet inclusion) on PPR, pellet durability index (PDI), and hot pellet temperature (HPT), while utilizing three different pellet die thicknesses (PDT, 32mm, 38mm, and 45mm). Feed was manufactured in a 2 (AZM) x 3 (PDT) factorial in a Latin square design, utilizing a California Pellet Mill conditioner and pellet mill, with 653kg experimental unit batches. The inclusion of AZM increased overall PPR by 7.9% ($P < 0.001$) and decreased PDI by 1.6 percentage points ($P < 0.001$). As PDT increased (32mm to 38mm/45mm), PPR decreased by 28.5% ($P < 0.001$), and PDI increased by 10.1 percentage points ($P < 0.001$). The interaction of AZM and PDT influenced HPT ($P = 0.007$), with AZM reducing HPT for the 32mm and 38mm PDTs; however, no difference was observed for the 45mm PDT. Linear contrast analysis showed that PPR increased by 5.0, 7.9, and 11.8% when comparing AZM treatments to their respective PDT controls (All PDT; $P < 0.001$). In conclusion, AZM increased pellet production rate across pellet die thickness and decreased pellet durability index, albeit to the extent that would likely not affect broiler performance.

Keywords: Azomite, Pellet Die Thickness, Pellet Production Rate, Pellet Durability

DESCRIPTION OF PROBLEM

Feed ingredient and manufacture costs constitute approximately 60-70% of the input cost in rearing meat-type poultry (McKinney and Behnke, 2007; Pope et al., 2018; Boltz et al., 2021). Therefore, economic profit may be largely influenced by feed manufacturing efficiency. Broiler diets are almost exclusively pelleted to improve feed flowability and handling and decrease nutrient segregation and selective consumption, thus increasing performance (Lilly et al., 2011; Glover et al., 2016; Boltz et al., 2021). Later phases of meat bird production necessitate large quantities of pelleted feed to meet increased feed intake and favor a high pellet production rate **(PPR)**. High PPR often is associated with poor pellet quality. Pellet production rate and pellet durability index (**PDI**), a standard method to assess pellet quality, can also be affected by many factors such as mixer added fat, conditioning time and temperature, the particle size of the feed, die specifications, and diet formulation (Fahrenholz, 2005; Buchanan and Moritz, 2009; Loar et al., 2010; Glover et al., 2016).

As poultry production costs continue to increase due to increased feed ingredient prices, cheaper feedstuffs have become more desirable to nutritionists and researchers alike. One such by-product alternative feed ingredient is dried distillers grains with solubles (**DDGS**). Tillman and coauthors (2020) reported a PPR decrease of 4.6% and a PDI decrease of 1.2 percentage points when formulating eight percent DDGS in the diet. Loar and coauthors (2010) also demonstrated a 7.6 and 12.3 percentage point decrease in PDI when including 15 and 30% DDGS in broiler diets, respectively. The authors of that experiment attribute the decreased PDI to the oil component of DDGS and adding lubrication in the pellet die. Inorganic phosphate sources have been used in poultry formulations for their mineral content and abrasive properties that improve PPR (Behnke, 1981; Wamsley et al., 2012). However, the use of these phosphorus sources has declined in recent as the use of exogenous phytases has continued to increase. This trend has likely been due to the increased cost and decreased availability of rock phosphates, as well as environmental concerns pertaining to the unavailable phytate phosphorus that is found in corn and soybean meal-based diets (Ennis et al., 2020; Gulizia et al., 2022). Therefore, other solutions must be found to achieve the same feed manufacturing benefit (Bowen et al., 2022).

Azomite (AZM, Azomite Mineral Products, Nephi, UT), is one such alternative ingredient that has been previously shown to increase PPR. This product originates from a solidified volcanic ash that is composed of a large portion of non-crystalline composites. Azomite is also characterized as a *dacitic tuff breccia* composition but is not strictly a dacite (Boltz et al., 2021). It is presumed that AZM affects feed manufacture by two mechanisms: the coarse fraction's irregular shape and angularity causes pellet die scouring that clears the pellet die channel of residue and the fine fraction interacts with heat and water, added through conditioning, and acts as a lubricant in the pellet die interior. Boltz and coauthors (2021) reported a PPR increase of 6.8% ($P < 0.001$) and a 0.75 percentage point decrease in PDI ($P < 0.001$) when including AZM at 0.25%. Also, in a follow-up study, Boltz and coauthors (2023) found that the inclusion of the separate fractions of AZM, coarse and fine, independently provided no increase in PPR when compared to the control ($P > 0.05$). However, the combination of the two fractions provided an increase in PPR when compared to the control (6.8%) and course fraction (5.0%; $P < 0.05$).

Another important factor that affects feed manufacture is the die specification, specifically, the length-to-diameter ratio (**L:D**), and both portions of this ratio can influence feed manufacture. Pellet die thickness (**PDT**), or length, relates to the effective thickness of the pellet die, and the diameter relates to the diameter of the pellet die channel for extrusion. Little research has been done on the effects of PDT on feed manufacturing. Behnke (1990) found that increasing the PDT from 38.1 to 50.8mm increased the PDI of corn and soybean meal-based diets by 19.8 percentage points and decreased PPR by 34.9% (Behnke, 1994). Pope et al. (2020) also found that decreasing the L:D from 10.0 to 8.0 decreased PDI by 6.2 percentage points and energy consumption per ton by 32.0%. Azomite has not been previously tested in conjunction with varying pellet die thicknesses and a constant pellet die channel diameter.

Therefore, the objective of the study was to determine the effect of a *dacitic tuff breccia* (Azomite), at an inclusion rate of 0.25%, in corn, soybean meal, and DDGS-based diets while utilizing either a 32mm x 4.5mm (7.1 L:D), 38mm x 4.5mm (8.4 L:D), or 45mm x 4.5mm (10.0 L:D) pellet die, on PPR, PDI and hot pellet temperature (**HPT**). The a priori hypothesis was that AZM would interact with the PDT to increase PPR as the L:D ratio increased.

MATERIALS AND METHODS

Diet Formulation and Batching

A corn, soybean meal, and DDGS-based finisher broiler diet was formulated using 90% of the lysine to amino acid ratios recommended by Tillman and Dozier (2013) (Table 1). This diet formulation was consistent with past research and facilitated nutrient detriment observation for pelleted feed fed to broilers. (Boltz et al., 2021; Bowen et al., 2022; Boltz et al., 2023). In total, 23,558 kg of a master batch formulation, with 0.25% less corn and only 1% mixer added fat, were batched and mixed. Following mixing, the feed was weighed into bags, each totaling 22.7 kg, and then allocated equally between 36 pallets (653 kg per pallet). Prior to pelleting, two experimental diets (Control and 0.25% AZM) were created by adding either the remaining corn or AZM. Ground corn and AZM were incorporated into approximately 1 kg of the master batch using a Univex paddle mixer for five minutes (Univex Floor Paddler Mixer, Model: M12B, Univex Corporation, Salem, NH), and subsequently mixed into the remaining master batch in a one-ton, vertical screw mixer, Easy Automation Inc. Modular Feed Processor (Easy Automation Inc., Welcome, MN).

Feed Manufacture

All feed was pelleted at the West Virginia University pilot feed mill located in Morgantown, WV. Pelleting was completed in 11 days of similar ambient temperature (22.95 ±

0.65˚C) to reduce the production variability. Treatments were arranged in a 2 (AZM; 0 or 0.25%) x 3 (PDT; 32, 38, or 45mm) factorial, with the diameter of the pellet being produced held constant among the pellet dies (4.5mm). Feed manufacture was conducted in a Latin Square design over six blocks or replicates of time. This experimental design allowed for each treatment to be manufactured in each possible position and reduced the chance of confounding bias of treatment associated with run order. Feed was pelleted using a California Pellet Mill (**CPM**) 40 HP pellet mill, conditioner, hygenizer, and CPM chrome plus (stainless steel; neutral hardened) pellet dies (Master Model Pellet Mill, CPM, Crawfordsville, IN). Steam pressure was held at a constant 276 kpa prior to entering the conditioner, the pellet mill load was controlled within a range of 40-43% of its maximum load amperage, and the conditioning temperature was maintained at 80°C for 30 seconds. While the hygenizer was not utilized to increase heated retention time, the feed still passed through as there is no bypass in West Virginia University's pilot feed mill system.

Once steady-state production was achieved, pellet mill motor load, feeder rate, conditioning temperature, and initial mash temperature were monitored via a Beta Raven programable logic control system and were recorded (PLC, 120V #10113354; Beta Raven Automation Solutions, St. Charles, MO). The authors define steady-state production as achieving the maximum pellet production rate while maintaining the target conditioning temperature within the motor load percentage range. Before each day of manufacture, a high-fiber ruminant diet, totaling 227 kg, was used to warm up the pellet mill's internal components and die. Following the pelleting of the high-fiber diet, experimental feed manufacture began by conveying each diet, in the Latin square design order, into a surge bin above the pellet mill. Here, six mash samples were taken evenly throughout conveyance and later composited to be sent for nutritional analysis at the Missouri Feed Labs (Experimental Station Chemical Laboratories, University of Missouri-Columbia, Columbia, MO). Nutritional analyses consisted of proximate analysis, Ca, total P, acid detergent fiber, and neutral detergent fiber. Post-steady-state pelleting, six 500 g samples of pellets were taken immediately after pellet die extrusion and cooled using a cheesecloth draped over a large agricultural fan. This procedure follows the methodologies of Reese et al. (2017), where ambient air was pulled across the pellets for a standardized 12 minutes to cool and remove moisture prior to sampling. These techniques have been shown to limit potential nutrient segregation and pellet degradation post-pelleting (Reese et al., 2017). The composited cooled pellet samples were also sent for similar nutritional analyses as the mash samples. Mixed-mash and cooled pellet nutrient analysis, as well as PLC, PPR, PDI, and HPT data, were collected for each replicated batch of manufactured feed.

Pellet production rate and HPT samples were collected once steady-state production was achieved. These measurements were collected four times equally spread throughout steady-state production. The PPR was measured by collecting all pelleted feed extruded directly from the pellet die for 60 sec and recording the resulting weight. Hot pellet temperature data was collected by filling an insulated thermos with pellets collected directly after extrusion, immediately closing the lid, and using an 80PK-24 temperature probe attached to a thermocouple to measure temperature (Fluke 51 II, Everette WA). Approximately one kilogram of pellets was collected directly from the end of the cooling deck to be analyzed for pellet durability index (PDI). The pellet durability index was determined 24 hours post-manufacture using a New Holmen Pellet Tester (NHP 100) (New Holmen portable Pellet Durability Tester, Lignotech USA Inc., Rothschild, WI). Prior to PDI analysis, all pelleted samples were sieved using a No. 5 W.S. Tyler testing sieve to remove confounding fine fractions from the sample (U.S.A Standard Test Sieve. No. 5 W.S. Tyler. Mentor, OH). One hundred grams of sieved pellets were added to the New Holmen Pellet Tester's perforated chamber, and forced air was applied for 30 sec to simulate typical auguring and transportation following manufacture. After the application of forced air, the remaining sample was removed and weighed, and the resulting weight was converted to a percentage. The pellet durability index was analyzed in triplicate for each replicate of manufactured feed. There was no attempt to correct for the PDI produced in this experiment. Based on the protocol, the observed PDI was a result of modifying the PDT and AZM inclusion rate while maintaining the motor load percentage at 40-43% of the maximum motor amperage throughout the production of each treatment in each replication.

As a supplement to the analytical data collected in this experiment, descriptive data, in the form of pellet die channel pictures, were collected during the sixth replication of manufacture to provide a visual representation of the influence of AZM on pellet die residue. Following the completion of each batch of feed, one pellet was removed from the pellet die, and a picture of the pellet die chamber was taken utilizing a Vividia GB-460 Gun barrel Borescope (Figures 1 and 2).

Statistical Analysis

Variables were analyzed in a 2 (AZM) x 3 (PDT) factorial arrangement in a Latin square design over six complete blocks of feed manufacture. The experimental unit was one 653 kg batch of feed. The blocking criteria for manufacture were the days of manufacture, and each treatment was manufactured at a different position in the pelleting order. Data were analyzed in a mixed-model analysis using the GLIMMIX procedure of SAS (SAS Institute, 2017), and a significance level was set at $P < 0.05$. The factorial arrangement was performed to compare the main effect means and determine interactions between AZM inclusion rate and PDT. Contrasts of treatments within the same pellet die thickness were completed to evaluate the effect of AZM within a single PDT across all PDTs utilized within this experiment. Tukey's HSD was utilized to further examine multiple comparisons if the interaction was significant and to analyze differences within the linear contrasts.

RESULTS AND DISCUSSION

Nutrient analyses for mash feed, cooled pellets, corn, soybean meal, and DDGS are demonstrated in Table 2. Based on these nutrient analyses, the experimental diets were of comparable composition and had high fiber content due to the inclusion of 5% DDGS.

Feed Manufacture, PPR, PDI, and HPT

Environmental feed manufacture variables measured during production, and averaged across treatment replications, were as expected due to planned methodologies (Table 3). Based on the data for ambient temperature, ambient humidity, mash temperature, and hygenizer temperature during manufacture, the authors can assume that feed manufacture metrics were not influenced by confounding effects. Therefore, feed manufacturing results were assumed to be caused by the inclusion of AZM and PDT.

The main effects and interactions are illustrated in Table 4. The 0.25% inclusion of AZM in the diet increased PPR by 7.9% (0.699 to 0.754 Mt/hr; $P < 0.001$). Azomite also caused a 1.6 percentage point decrease in PDI (90.6 to 89.0%; $P < 0.001$). The authors attribute the increase in PPR to the scouring and lubrication properties that AZM has been hypothesized to have and the subsequent decrease in feed retention time in the pellet die chamber. These findings agree with the results from Boltz and coauthors (2021), who saw an increase in PPR of 6.8% (1.049 to 1.120 Mt/hr) with the inclusion of 0.25% AZM ($P < 0.001$). Tillman and coauthors (2020) also saw an increase of 3.3% (0.657 to 0.679 Mt/hr; $P < 0.001$) in PPR and no difference in PDI ($P = 0.338$) when using the same inclusion rate of AZM. However, Boltz et al. (2021) and Tillman et al. (2020) utilized pellet dies with L:D ratios of 8.1 and 8.0, respectively, whereas the current study results were pooled across L:D ratios of 7.1, 8.4, and 10.0. The pellet durability index decrease can likely be attributed to the proposed abrasive and lubricating actions of AZM, presumably leading to less exposure to the frictional heat and pressure within the pellet die channel. The decreased exposure likely led to less starch gelatinization and protein gelation as a large portion of these chemical reactions has been deduced to occur at the pellet die (Pope et al., 2020). Overall, the PDI difference observed in the current study would likely not influence broiler performance based on findings that a 10-percentage point difference is needed to recognize performance differences; specifically, FCR decreases (Glover et al., 2016; Moritz, 2021).

Pellet die thickness influenced both PDI and PPR. PDI increased 10.1 percentage points as PDT increased from 32mm (83.1%) to 38mm/45mm (93.2%; P <0.001). Once again, the increase in PDI was attributed to a longer exposure time of feed to the heat and pressure associated with the pellet die, promoting more chemical reactions that stabilize the pellet being produced (Behnke, 1990). In complement, PPR was decreased by 28.4% (0.897 to 0.642 Mt/hr; P < 0.001) as PDT increased from 32mm to 38mm/45mm. The observed decrease in PPR is likely associated with the increased friction and shear between the feed and the pellet die, thus retaining the feed in the chamber for a longer period (Pope et al., 2020). Both findings are in agreeance with other experiments focusing on PDT. Behnke (1990) found that increasing PDT from 38.1 to 50.8mm increased PDI by 19.8 percentage points (74.5 to 94.3%) and decreased PPR by 34.8% (0.994 to 0.647 Mt/hr) in corn and soybean meal-based diets. Similarly, Smith and coauthors (2021) found that increasing PDT from 19.1 to 32mm increased PDI by 18.3 percentage points (32.9 to 51.2%). Contrary to the main effects of AZM, the difference in PDI seen in this experiment would likely influence broiler performance as it meets the 10-percentage point threshold (Glover et al., 2016; Moritz, 2021). Therefore, increasing the PDT from 32mm to 38mm/45mm may decrease PPR and provide a PDI that would improve broiler performance assuming nutrient availability was not decreased in the process.

An interaction of AZM and PDT was recognized for HPT (P = 0.007; Table 4). AZM reduced HPT for the 32 and 38mm pellet dies but not for the 45mm die. The authors attribute this finding primarily to the abrasive and lubricating properties of AZM that may have decreased the friction and shear between the feed and pellet die. The supposed decreased friction and shear could then decrease the heat transfer from the pellet die to the pellet itself, thus decreasing the observed HPT. The authors speculate that the 45mm pellet die generated too much heat from the friction and shear between the feed and pellet die for AZM to decrease the HPT. However, the observed decrease in HPT (2.6°C), from the highest observed treatment (45mm + 0.25% AZM: 82.89°C) to the lowest observed treatment (32mm + 0.25% AZM: 80.29°C), may not have practical significance to poultry producers. Bowen and coauthors (2022) found that AZM interacts with rock phosphate type to increase the apparent ileal amino acid digestibility of Cys (P = 0.034), and the trended towards increasing the digestibility of Lys (P = 0.058), Ile (P = 0.070), and Val (P = 0.097). The authors of that study attributed the increase in digestibility to decreased pressure and heat caused by the pellet die chamber. Therefore, PDT and AZM may have influences on the apparent ileal amino acid digestibility of various essential amino acids. However, Truelock and coauthors (2019) saw that when increasing PDT from 22mm to 32mm, the increase provided no influence on phytase stability (P = 0.85); moreover, as conditioning temperature increased (165 to 185°C) a linear decrease in phytase activity was seen (P < 0.01). In contrast, Pope and coauthors (2020) found that increasing PDT from 35mm to 44mm decreased the xylanase recovery rate when comparing conditioned mash to pellets (60.1% to 22.9% recovery; P = 0.003), and the change in temperature from the conditioned mash to pellets was 4.26 to 7.22°C (P = 0.001) for the respective PDTs. Therefore, the effect of PDT on enzyme recovery rates may fluctuate based on the enzyme, enzyme source, or its thermostability throughout feed manufacture. Currently, there is no published research on AZM and its influence on enzyme recovery rates.

Contrasts were performed to observe potential differences between the addition of AZM within each PDT (Table 5). Analysis of the 32mm die showed that AZM decreased PDI by 2.3 percentage points (84.3 to 82.0%; P < 0.001) and increased PPR by 5.0% (0.875 to 0.919 Mt/hr; P < 0.001). A decrease of 1.2 percentage points in PDI (93.9 to 92.7%; P = 0.039) and an increase of 7.9% for PPR (0.630 to 0.680 Mt/hr; P < 0.001) was observed with the addition of AZM for the 38mm die. AZM added to the 45mm die decreased PDI by 1.3 percentage points (93.7 to 92.4%; P = 0.030) and an increased PPR by 11.8% (0.593 to 0.663 Mt/hr; P < 0.001).

The increases in PPR (32mm: 5.0%, 38mm: 7.9%, and 45mm: 11.8%) when AZM was included may be attributed to the potential scouring and lubricating properties of AZM. Figures 1 and 2 are pictures of the pellet die channel for the 45mm Control and 45mm + 0.25% AZM treatments respectively. Based on these pictures, the authors can infer that the inclusion of AZM may have decreased the amount of residue in the pellet die chamber, thus increasing PPR. Similar subjective differences were seen when comparing the Control to the AZM treatments for the 32 and 38mm treatments.

Jones and coauthors (2022) found that when including AZM at 0.25% of the diet and using a 4.0mm x 35 mm pellet die (8.7 L:D ratio), PPR increased by an average of 1.77% over two days of production. These data do not support the results in the current study, especially compared with the 4.5mm x 38mm pellet die (8.4 L:D). The variation in PPR may be linked to the difference in the pellet die diameter. The 0.5 mm decrease in pellet diameter in the Jones et al. study may have generated too much friction and pressure to overcome with AZM. The decrease in PDI in the current study (32mm: 2.3, 38mm: 1.2, and 45mm: 1.3 percentage points) may be explained by the increase in PPR and reduced residual time of the feed in the pellet die, as well as AZM's hypothesized properties. Saensukjaroenphon and coauthors (2019) found that increasing PDT from 32mm to 45mm and from 45mm to 51mm increased PDI by 22.8 and 9.4 percentage points, respectively when pellet diameter was held to a constant of 4.8mm. The authors of that study attributed the increase in PDI to increased retention time within the pellet die and subsequent gelatinization and gelation.

CONCLUSIONS AND APPLICATION

1. The inclusion of AZM, at 0.25% of the diet, increased PPR (7.9%) and decreased PDI (1.6 percentage points). Although PDI decreased with the inclusion of AZM, the magnitude of the decrease is not expected to influence broiler performance.
2. Azomite® included at 0.25% in feed manufactured with 32mm, 38mm, and 45mm pellet die thicknesses increased pellet production rate by 5.0, 7.9, and 11.8%, respectively. However, no interaction between AZM inclusion and PDT was observed in this experiment.

3. Increasing PDT from 32mm to 38mm/45mm resulted in reduced PPR (28.4%), and improved PDI (10.1 percentage points).

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TABLES AND FIGURES

Table 1. Diet compositions and calculated nutrient values of the Control and Azomite treatments

Ingredient, %	Control	Azomite
Corn	53.95	53.70
Soybean meal (45.7% CP)	32.97	32.97
DDGS[1]	5.00	5.00
Soybean Oil[2]	4.82	4.82
Dicalcium Phosphate	1.70	1.70
Limestone	0.74	0.74
Sodium Chloride	0.27	0.27
L-Methionine	0.19	0.19
Vitamin/Mineral Premix[3]	0.25	0.25
L-Lysine HCl	0.008	0.008
L-Threonine (80%)	0.003	0.003
Sodium Bicarbonate	0.1	0.1
Azomite	0	0.25
Calculated Nutrient Values, %		
ME (kcal/kg)	3,115	3,115
Crude Protein	19.55	19.55
Digestible Lysine	1	1
Digestible TSAA	0.77	0.77
Digestible Methionine	0.49	0.49
Digestible Threonine	0.68	0.68
Digestible Tryptophan	0.22	0.22
Calcium	0.76	0.76
Available Phosphorus	0.38	0.38
Sodium	0.17	0.17

[1] Dried Distillers Grains with Solubles

[2] Only 1.00% of the 4.82% Soybean Oil was added at the mixer

[3] 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. Lab analyzed nutrient concentrations of the Control and Azomite Diets, and the major ingredients utilized

Diet\Nurient[1]	Crude Protein[2]	Crude Fat[3]	Crude Fiber[4]	Ash[5]	Ca[6]	P[7]	NDF[8]	ADF[9]	Moisture[10]
Control	21.55	2.39	2.64	5.56	0.89	0.72	9.03	4.22	12.15
AZM	21.67	2.49	2.65	5.58	0.85	0.70	8.98	4.48	12.23
Corn	10.60	2.85	1.57	1.10	-	-	-	-	13.88
Soybean Meal	45.76	1.33	3.52	6.27	-	-	-	-	11.67
DDGS	30.55	4.63	8.54	5.03	-	-	-	-	12.41

[1] All nutrient results are presented as percentages of the total nutrient profile.

[2] Crude protein was determined using the LECO method (AOAC Official 992.23/990.03).

[3] Crude fat was determined using petroleum ether in a Soxhlet apparatus method (AOAC Official Method 920.39).

[4] Crude fiber was determined by extracting fiber residue to be weighed, then ignited and reweighed to calculate the loss on ignition of residues (AOAC Official Method 973.18).

[5] Ash was determined by heating the sample for 2 h at 600°C (AOCS Official Method Ba 5b-68).

[6] Total calcium was determined by atomic absorption spectroscopy (AOAC Method 968.08).

[7] Total phosphorus was determined by colorimetric spectroscopy (AOAC Method 965.17).

[8] Neutral Detergent Fiber was determined using the Holst Filtration Apparatus method (JAOAC 56, 1352-1356).

[9] Acid Detergent Fiber was determined by the reflux apparatus method (AOAC Official Method 973.18).

[10] Moisture was determined by the freeze-drying method (AOAC Official Method 934.01).

Table 3. Descriptive data of constant variables held throughout feed manufacture utilizing a 40 horsepower California Pellet Mill at the WVU Poultry Feed Mill[1]

Treatment	Ambient Temperature (°C)	Ambient Humidity (%)	Mash Temperature (°C)	Hygenizer Temperature[2] (°C)	Conditioning Temperature[3] (°C)	Conditioning Time (s)	Mill Load[4,5] (%)
32mm Control	22.31	69.50	30.83	73.80	82	30	40-43
32mm + 0.25% AZM	22.50	69.17	31.21	73.70	82	30	40-43
38.1mm Control	22.69	69.83	31.48	71.57	82	30	40-43
38.1mm + 0.25% AZM	23.15	67.50	31.11	71.85	82	30	40-43
45mm Control	23.61	69.00	31.29	71.02	82	30	40-43
45mm + 0.25% AZM	23.52	68.67	31.21	70.93	82	30	40-43

[1] Ambient temperature, ambient humidity, mash temperature, and hygenizer temperature values are the averages of the values taken over the 6 replications.

[2] Hygenizer temperature was measured as the reading from the hygenizer temperature probe at the time of sample collection. This temperature was taken during a steady state position.

[3] Conditioning temperature was measured as the reading from the conditioner temperature probe at the time of sample collection. This temperature was taken during a steady state position.

[4] A 100% motor load was based on FLA (full load amps) which was 47 amps based on the pellet mill motor nameplate.

[5] Mill load fluctuated throughout this range during production.

Table 4. The response of AZM and PDT on measured feed manufacture variables and the corresponding multiple comparison and factorial statistics.

Manufacture Treatment	Pellet Durability Index[1] (%)	Hot Pellet Temperature[2] (°C)	Production Rate[3] (Mt/hr)[4]
32mm Control	84.267	80.926^{cd}	0.875
32mm + 0.25% AZM	82.003	80.289^{d}	0.919
38mm Control	93.931	82.227^{ab}	0.630
38mm + 0.25% AZM	92.703	81.345^{bc}	0.680
45mm Control	93.698	82.833^{a}	0.593
45mm + 0.25% AZM	92.406	82.889^{a}	0.663
Treatment SEM	0.415	0.153	0.007
Treatment P-value	<0.001	<0.001	<0.001
Azomite			
0%	90.632^{a}	81.995	0.699^{a}
0.25%	89.037^{b}	81.508	0.754^{b}
Azomite SEM	0.240	0.088	0.004
Azomite P-value	<0.001	<0.001	<0.001
Die Size			
32mm	83.135^{b}	80.608	0.897^{a}
38mm	93.317^{a}	81.786	0.655^{b}
45mm	93.052^{a}	82.861	0.628^{b}
Die Size SEM	0.294	0.108	0.005
Die Size P-value	<0.001	<0.001	<0.001
Interaction SEM	0.866	0.298	0.017
Interaction P-value	0.379	0.007	0.113

[1] Pellet durability was measured using the New Holmen Pellet Tester, where 100 grams of pelleted samples were subjected to airflow within a perforated chamber for 30 seconds.

[2] Hot pellet temperature was measured four times periodically throughout feed manufacture once steady state production was achieved.

[3] Production rate was measured four times periodically throughout feed manufacture once steady state production was achieved.

[4] Metric tons per hour

Table 5. The response of AZM within a single PDT on measured feed manufacture variables and the corresponding contrast statistics.

Manufacture Treatment	Pellet Durability Index[1] (%)	Hot Pellet Temperature[2] (°C)	Production Rate[3] (Mt/hr)[4]
32mm Control vs. 32mm + 0.25% AZM			
Control	84.267^{a}	80.926^{a}	0.875^{b}
0.25% AZM	82.003^{b}	80.289^{b}	0.919^{a}
SEM	0.294	0.108	0.005
P-value	<0.001	0.003	<0.001
38mm Control vs. 38mm + 0.25% AZM			
Control	93.931^{a}	82.227^{a}	0.630^{b}
0.25% AZM	92.703^{b}	81.345^{b}	0.680^{a}
SEM	0.294	0.108	0.005
P-value	0.039	<0.001	<0.001
45mm Control vs. 45mm + 0.25% AZM			
Control	93.698^{a}	82.833	0.593^{b}
0.25% AZM	92.406^{b}	82.889	0.663^{a}
SEM	0.294	0.108	0.005
P-value	0.030	0.797	<0.001

[1] Pellet durability was measured using the New Holmen Pellet Tester, where 100 grams of pelleted samples were subjected to airflow within a perforated chamber for 30 seconds.

[2] Hot pellet temperature was measured four times periodically throughout feed manufacture once steady state production was achieved.

[3] Production rate was measured four times periodically throughout feed manufacture once steady state production was achieved.

[4] Metric tons per hour.

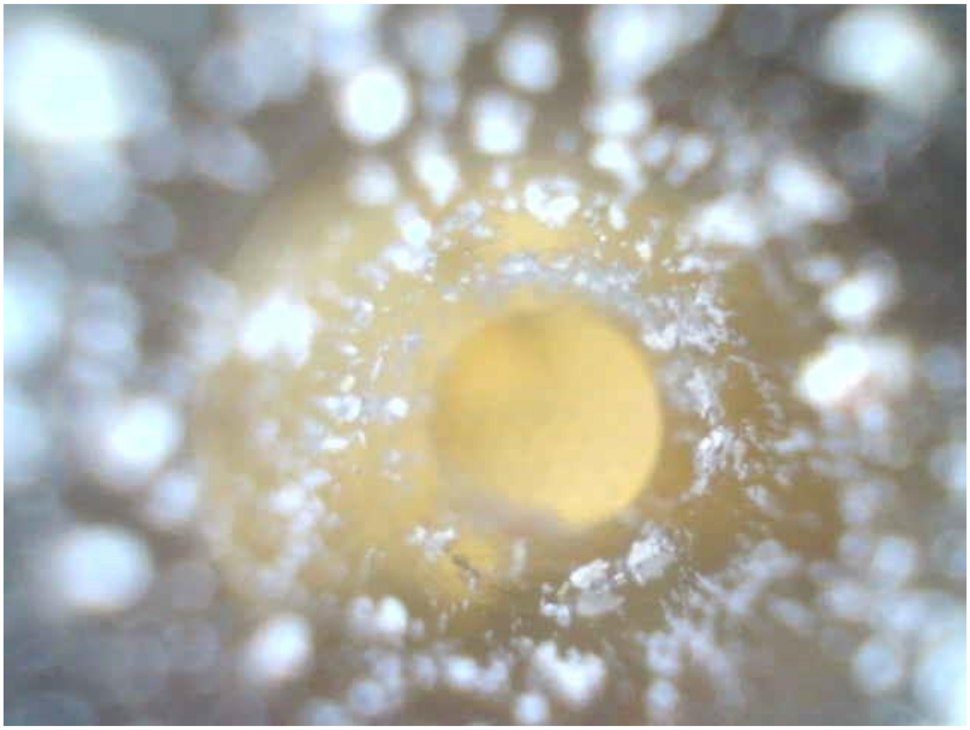

Figure 1. Pellet Die Chamber Following Manufacture of the 45mm Control Treatment.

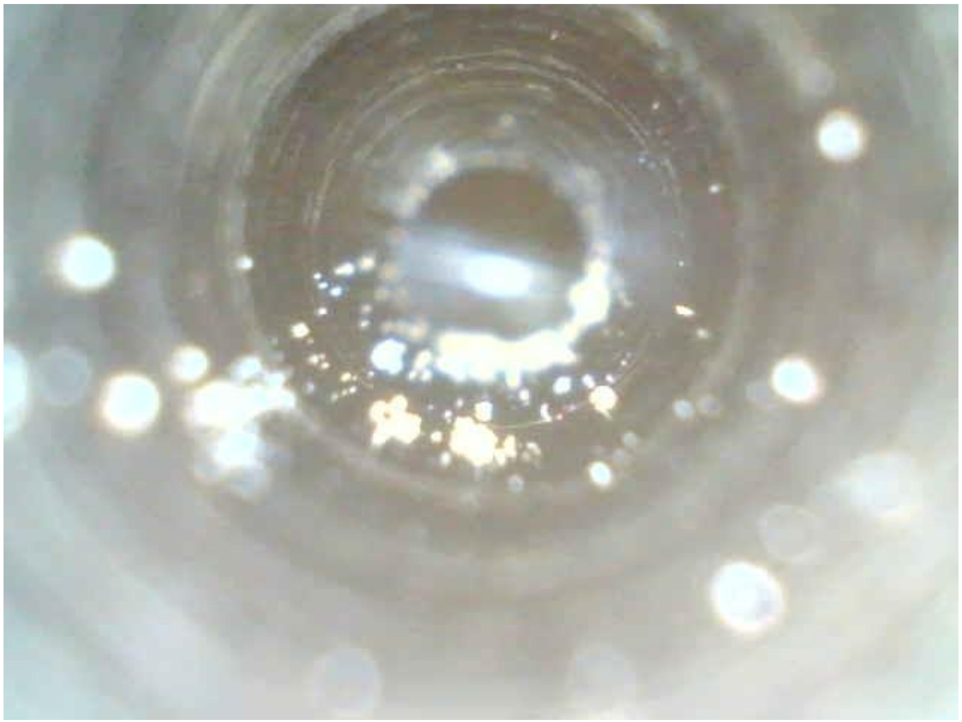

Figure 2. Pellet Die Chamber Following Manufacture of the 45mm + 0.25% AZM Treatment

Chapter Four

Pellet Die Thickness and the use of a Throughput Agent Interacted to Demonstrate that High Frictional Heat Increased Apparent Ileal Amino Acid Digestibility, but did not Influence Trypsin Inhibitor Activity or Male Broiler Performance

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Primary Audience: Feed Manufacturers, Nutritionists, Researchers

SUMMARY

The inclusion of Azomite® (AZM) in broiler diets containing dicalcium phosphate has been shown to increase apparent ileal amino acid digestibility (AIAAD). These findings are likely due to die-scouring and lubrication properties that decreased the frictional heat exposure of feed. Past research indicates that modifying pellet die thickness (PDT) affects the frictional heat exposure of feed. Therefore, it was hypothesized that PDT and AZM would interact to influence AIAAD and broiler performance. The study's objective was to determine the effect of AZM (0.0% or 0.25%) and PDT (32 and 45 mm with a common pellet diameter) on broiler performance and AIAAD from 0 to 21 days of age. Live performance did not differ due to the interaction or main effects ($P > 0.05$). However, AIAAD was influenced by AZM and PDT interactions ($P < 0.05$), with 11 amino acids demonstrating increased digestibility in the 45mm control treatment. The AIAAD increase was likely not enough to influence performance. The increased frictional heat was presumed to deactivate trypsin (TI) and chymotrypsin inhibitors (CTI), ultimately increasing AIAAD. Quantitative analysis of TI and CTI activity, utilizing a novel assay based on the current American Oil Chemists' Society and the American Association of Cereal Chemists International accepted procedure, showed no practically influential amount of either inhibitor before or after pelleting. The authors, therefore, speculate that the increased AIAAD was due to cell lysis of the corn aleurone layer via increased frictional heat exposure of the 45mm PDT and the absence of AZM.

Keywords: Azomite, Pellet Die Thickness, Trypsin Inhibitor, Aleurone Layer

DESCRIPTION OF PROBLEM

Feed and feed manufacture are known to constitute approximately 60 to 70% of the input cost associated with rearing broilers (McKinney and Behnke, 2007; Pope et al., 2018; Boltz et al., 2021; Knarr et al., 2023). Improvements in feed manufacture, while being the smaller factor of the proposed 60-70%, can still have positive or negative ramifications on broiler performance. In the US, broiler diets are almost exclusively pelleted to decrease selective consumption, decrease ingredient segregation, and increase productive energy, as well as increase handling characteristics and flowability (Lilly et al., 2011; Glover et al., 2016; Boltz et al., 2021). However, modifying pelleting parameters can also affect nutrient digestibility, exogenous enzyme activity, and digestive inhibitor activity (Saunders et al., 1969; Truelock et al., 2019; Milani et al., 2022).

Past research has shown that modifying pelleting parameters, specifically increasing pellet die thickness (**PDT**), leads to increased hot pellet temperature (**HPT**), while the addition of Azomite® (**AZM**), a ground, solidified volcanic ash used as a throughput agent, has been shown to interact with PDT to decrease HPT (Knarr et al., 2023; $P < 0.001$). Increased HPT can potentially lead to nutrient degradation and deactivation of exogenous enzymes. Pope and coauthors (2020) found that increasing PDT from 35.2mm to 44mm, while controlling pellet diameter to 4.4mm, decreased xylanase activity by 37.2 percentage points (60.1% to 22.9%; $P < 0.01$). In that same experiment, the change in temperature from conditioned mash to pellets increased by 1.52°C under the same modification in pelleting characteristics (4.74 to 6.26°C; $P = 0.007$). Similarly, high temperatures and shear forces caused by pelleting, in combination with low moisture, have been shown to favor Maillard reactions between free aldehyde groups from reducing sugars and the epsilon-amino group of Lys (Thomas et al., 1998; Abdollahi et al., 2013; Boroojeni et al., 2016). This can create products that are indigestible by poultry, thus rendering a portion of Lys, the second limiting amino acid, inaccessible for use in growth (Martinez-Amezcua and Parsons, 2007; Boroojeni et al., 2016; Lynch et al., 2023). Similar to Lys and the Maillard reaction, cysteine, arginine, threonine, and serine are known to be heat-labile amino acids, where high temperatures can cause degradation (Papadopoulos, 1988; Boroojeni et al., 2016). Bowen and coauthors (2022) found an inorganic phosphate source x AZM interaction on the apparent ileal amino acid digestibility (**AIAAD**), with the dicalcium phosphate + 0.25% AZM treatment promoting the highest percent digestible Cys (P = 0.034). In addition, the authors observed a trend of increased AIAADs for Lys (P = 0.058), Ile (P = 0.070), and Val (P = 0.097) with the same interaction-treatment tendency (Bowen et al., 2022). The authors attributed the increase in AIAAD to the potential die-scouring and lubrication properties of AZM. Hence, AZM decreased the friction and pressure forces of the pellet die on the feed, which decreased the number of conformational changes in denatured intact protein chains, ultimately increasing AIAAD. Therefore, the addition of AZM, in combination with varying PDTs, may lead to increased AIAAD of various amino acids via reducing denatured protein chain conformational changes and/or irreversible reactions, thus improving broiler performance.

Other nutritional factors that can influence broiler performance are trypsin inhibitors (**TI**) and chymotrypsin inhibitors (**CTI**). Erdaw and coauthors (2016) note that natural TI and CTI are serine protease inhibitors that can be broken into two classes: Kunitz trypsin inhibitors (larger and more influential on trypsin) and Bowman-Brik inhibitors (smaller and inhibit both trypsin and chymotrypsin). These inhibitors are known to be in high concentrations in unprocessed and under-processed soybean meal, but are heat-labile (Clemente et al., 2007; Erdaw et al., 2016; Erdaw et al., 2017). Therefore, sufficient heat-processing of soybean meal, following the procedure of extracting the oil fraction of soybeans, results in soybean meal that is practically devoid of TI and CTI. However, in the case of under-processed soybean meal, these inhibitors may still be inactivated during the pelleting process of feed manufacture. Bergeron and coauthors (2018) found that pelleting typical corn and soybean meal-based poultry diets decreased trypsin inhibitor activity (**TIU**) by 14.2% (2750 to 2360 TIU/g) and increased methionine and threonine digestibility by

5.8% (P = 0.0001) and 3.4% (P = 0.0057) respectively. Therefore, increased pelleting stresses via increasing PDT, resulting in increased HPT, may function to deactivate TI and CTI in corn and soybean meal-based poultry diets. However, the addition of AZM, even with increased PDT, may decrease the extent of TI and CTI deactivation as AZM has been shown to decrease HPT (Knarr et al., 2023).

Therefore, the objective of this study was to determine the effect of feed pelleted using different inclusion rates of AZM (0 or 0.25%) and different PDT (32mm and 45mm; using a constant die diameter of 4.5mm) on Ross 308 broiler feed intake, live weight gain, feed conversion ratio, and AIAAD from 0 to 21 days of age.

MATERIALS AND METHODS

Diet Formulation and Batching

Feed for this experiment was manufactured in a previous experiment studying the effects of AZM and PDT on PPR, PDI, and HPT (Knarr et al., 2023). Feed from the 32mm Control, 32mm + 0.25% AZM, 45mm Control, and 45mm + 0.25% AZM treatments was obtained from the fifth replication of manufacture in that experiment and collected once steady-state production was achieved. Following collection of the feed, 136 kg of each treatment was reground into a fine crumble form utilizing a Badger Single-Pair Roller Mill (Badger Roller Mill, Model BN136). The fine crumble feed, from each treatment, was then reformulated to better meet the requirements of broilers from 0 to 21 days of age (Table 1). Titanium dioxide (TiO_2) was also added to the diets as an indigestible marker for AIAAD. The reformulated feed was mixed for five minutes in a Davis Precision Horizontal Batch Mixer to incorporate the added dry ingredients, and then another five minutes to incorporate the oil (Model: S-1, H.C. Davis Sons Manufacturing Company Inc., Bonner Springs, Kansas). Following reformulation, representative samples of each treatment were analyzed via proximate analysis and for total Ca and total P concentrations (Table 2). All nutritional analyses were performed at Missouri Feed Labs (Experimental Station Chemical

Laboratories, University of Missouri-Columbia, Columbia, MO). From the analyses, the authors confirmed that each treatment was of similar formulated nutritional content, thus performance differences can be deduced to come from the modified manufacturing variables.

Particle Size

The particle size of the mash diets presented in this experiment was measured using a WS Tyler Ro-Tap RX-29 Sieve Shaker. One hundred grams of representative sample, from each treatment, was placed in the Ro-Tap and subjected to 10 minutes of pressure in the enclosed sieves. Sieve sizes, from top to bottom, are as follows: 4.75 mm, 3.35 mm, 2.36 mm, 1.70 mm, 1.18 mm, 850 µm, 600 µm, 425 µm, 300 µm, 212 µm, 150 µm, 106 µm, and 75 µm. Once the time interval was completed, each sieve was removed and independently weighed, the contents removed, and reweighed to calculate the amount retained by each sieve. These values were then utilized to calculate the mean geometric particle size and standard deviation for each treatment (Table 3).

Broiler Rearing

Approximately six weeks following feed manufacture, reformulated feed from the 32mm control, 32mm + 0.25% AZM, 45mm control, and 45mm + 0.25% AZM treatments were fed to broilers to observe performance metrics and AIAAD. Ten replications, of ten male Ross 308 broilers, totaling 40 pens and 400 broilers, were fed for 21 days in raised wire cages under the IACUC Protocol #1602000612_R1.2. Upon placement, pen weights were measured and recorded, and blocked by weight (within 10 g of each other). Feed and water were provided for ad libitum consumption, and the weight of the feed given was recorded as provided and compiled upon the conclusion of the experiment. On day 21, pen weights were measured and recorded, and four birds were selected, at random, for lower ileal digesta collection to evaluate TiO_2 and amino acid content. Unconsumed feed was also collected on day 21, and the weight was recorded. Mortality numbers and weight were recorded when necessary throughout rearing. Upon conclusion of the experiment, all broilers were humanely euthanized via cervical dislocation and disposed of properly once all sample collections were completed. Data collected were utilized to calculate feed intake (**FI**), live weight gain (**LWG**), mortality corrected feed conversion ratio (**FCR**), and mortality percentage (**MORT%**).

Apparent Ileal Amino Acid Digestibility

As previously mentioned, on day 21, four broilers from each pen were subjected to lower ileal content collection. This process involved the removal of the small intestine from Meckel's diverticulum to the ileocecal-colonic junction, selecting the lower half of that segment, and flushing the contents into a clean, plastic container with deionized water. The digesta samples were then stored at -16°C until being dried in a forced draft oven at 60°C for 48-h. After drying, the digesta samples were once again stored at -16°C until being sent for analysis. These methodologies were adapted from the methodologies used by Angel et al. (2005). The digesta samples, as well as representative feed composites, were analyzed for the total concentration of Ala, Asp, Cys, Glu, Gly, Ile, Leu, Lys, Met, Pro, Thr, and Val. These samples were also analyzed for TiO_2 concentrations which were used as the indigestible marker. All analyses were performed at Missouri Feed Labs (Experimental Station Chemical Laboratories, University of Missouri-Columbia, Columbia, MO). The percentage of AIAAD was determined using the following formula:

$$\boldsymbol{AIAAD}\ (\%) = \left[\mathbf{1} - \left(\frac{\boldsymbol{TD_i}}{\boldsymbol{TD_0}}\right) \boldsymbol{x} \left(\frac{\boldsymbol{N_0}}{\boldsymbol{N_i}}\right)\right] \boldsymbol{x}\ \mathbf{100}$$

Where...

TD_i represents the concentration of TiO_2 in the diet in grams per kilogram of DM

TD_0 represents the concentration of TiO_2 in the ileal digesta in grams per kilogram of DM

N_i represents the concentration of amino acid in the diet in grams per kilogram of DM

N_0 represents the concentration of amino acid in the ileal digesta in grams per kilogram of DM

Trypsin and Chymotrypsin Inhibitor Analysis

Trypsin inhibitor activity was measured using the procedure described by Kim and Krishnan (2023). Briefly, finely ground feed powder (20 mg) was extracted with 1 ml of 10 mM NaOH on a vortex mixer for 10 min. The slurry was centrifuged at 16,000 g for 10 min and the resulting supernatant was saved and used for the enzyme assay. Feed extract, which resulted in 30 to 70% trypsin inhibition range, was added to a 1.5 mL Eppendorf tube containing 200 µl of assay buffer (50 mM-Tris-HCL, pH 8.2 and 20 mM $CaCl_2$), 500 µl of Nα-Benzoyl-L-arginine 4-nitroanilide hydrochloride (BAPNA; 0.4 mg/ml), and 200 µl of trypsin (20 µg/ml). The reaction was carried out for 10 min at 37 °C, terminated by the addition of 200 µl of 30% acetic acid, and absorbance at 410 nm was measured. Trypsin units inhibited was calculated as outlined earlier (Kim and Krishnan, 2023). Similarly, CTI activity was measured in a 1.5 mL Eppendorf tube containing 900 µl of assay buffer (100 mM-Tris-HCL, pH 8.0), 8 µl of α-chymotrypsin dissolved in 1 mM HCl solution (0.1 mg/ml) and feed extract and incubated at 37 °C for 5 min. Following this step, 80 µl of N-Succinyl-Ala-Ala-Pro-Phe p-nitroanilide (AAPF; 1 mg/ml) was added and incubated for another 10 min at 37 °C. The reaction was terminated by the addition of 500 µl of 30% acetic acid and absorbance at 410 nm was measured. Chymotrypsin inhibitor units were calculated as the amount of inhibitor that reduced the absorbance at 410 nm by 0.1 optical density.

Protein Extraction and Gel Electrophoresis

To extract total protein, feed formulations were ground to a fine powder with a mortar and pestle. Twenty mg of finely ground feed powder was extracted with 1 mL of sodium dodecyl sulfate (SDS)- sample buffer (60 mM Tris-HCl, pH 6.8, 2% SDS (w/v), 10% glycerol (v/ v), and 5% 2-mercaptoethanol (v/v). The slurry was boiled at 100 ◦C for 5 min followed by centrifugation at 15800xg for 5 min. The resulting supernatant was transferred to a new 1.5 mL plastic tube designated as the total feed protein fraction. Total feed proteins were then resolved by 13.5% SDS-PAGE gels using a Hoeffer SE 250 Mini-Vertical electrophoresis apparatus (GE Healthcare, Pittsburg, PA, U.S.A.). Electrophoresis was conducted at a constant 20 mA/gel until the tracking dye reached the bottom of the gel. After completion of electrophoresis, the gels were stained with Coomassie Blue R-250 solution overnight.

Immunoblot Analysis

Immunoblot analysis was performed following the procedure described earlier (Song et al., 2016). Briefly, feed proteins separated by SDS-PAGE gels were transferred to a nitrocellulose membrane and incubated overnight with soybean Kunitz trypsin inhibitor-specific polyclonal antibodies. Proteins reacting specifically with the soybean TI antibodies were detected using the SuperSignal West Pico kit (Pierce, Rockford, IL, U.S.A.).

Statistical Analysis

Performance variables and AIAAD values were analyzed in a 2 (AZM) x 2 (PDT) factorial arrangement in a randomized complete block design. The experimental unit was defined as one cage of 10 broilers. The blocks were defined as a complete set of all four adjacent treatment cages. The factorial arrangement was performed to compare the differences between the main effect levels (AZM or PDT) and the interactions between the two. Data were analyzed using the GLM procedure of SAS 9.4 (SAS Institute, Cary, NC 2017). Multiple comparisons of all treatments were analyzed post-hoc using Fisher's LSD. The alpha level of significance was set at $P \leq 0.05$, and a trend was defined as $P \leq 0.10$. Finally, linear contrasts were examined for treatment comparisons of interest.

RESULTS AND DISCUSSION

Broiler Performance

Multiple comparisons, factorial, and linear contrast analyses of the Ross 308 broiler performance did not provide any significantly different treatments for any of the performance metrics measured (FI, LWG, end bird weight, FCR, and MORT%) (Table 4). Similarly, trends associated with broiler performance effects were not recognized in this experiment ($P > 0.10$). The results of this portion of the study correspond with the findings of Bowen et al. (2022), who found no significant effect on end bird weight, MORT%, FI, LWG, or FCR with the addition of AZM ($P > 0.05$). Based on the particle size of the crumbled feed presented to the broilers in this study, the authors conclude that the feed form was not a confounding effect of this experiment (Table 3).

Amino Acid Digestibility

Nutritional analyses of lower ileum digesta, and subsequent statistical analysis, presented an interaction of AZM and PDT for AIAAD of Ala ($P = 0.018$), Asp ($P = 0.008$), Glu ($P = 0.007$), Gly ($P = 0.016$), Ile ($P = 0.013$), Leu (0.012), Lys ($P = 0.022$), Met ($P = 0.032$), Pro ($P = 0.045$), Thr ($P = 0.021$), and Val ($P = 0.013$) with the 45mm control treatment resulting in an increase of 1.4 to 4.8% numerical increase in AIAAD compared to the average of the other treatments (Table 5). Cys AIAAD was increased by 3.24 percentage points when PDT increased from 32 to 45mm (65.97 to 69.21; $P = 0.042$). However, the AIAAD of Cys was not significantly affected by the inclusion of AZM ($P = 0.169$). In contrast, Bowen and coauthors (2022) found that AZM interacts with rock phosphate type to increase AIAAD of Cys (1.1 percentage points; $P =0.034$) when comparing the dicalcium phosphate control treatment to the dicalcium phosphate + 0.25% AZM treatment. The authors also found a trend increase of AIAAD for Lys ($P = 0.058$), Ile ($P = 0.070$), and Val ($P =0.090$) from this interaction. Bowen and coauthors (2022) attribute the increase and trend to the potential pellet die scouring and lubrication properties of AZM decreasing the amount of friction between the feed and pellet die, thus sparing heat liable amino acid. However, similarly to the current study, the increase seen in AIAAD did not translate into improved broiler performance.

The authors of the current study hypothesized that increasing PDT would lead to decreased amino acid digestibility, especially for the heat liable amino acids Cys and Thr as well as Lys, due to the increased pressure, heat, and shear forces caused by the increased retention time of the feed within the pellet die potentially promoting more Maillard reactions and protein conformational changes. Similarly, the authors hypothesized the inclusion of AZM can decrease the influence of the aforementioned forces due to the proposed die scouring and lubrication properties associated with AZM, thus increasing AIAAD. However, the interaction recognized between AZM and PDT to increase the AIAAD with increased PDT and 0.0% inclusion of AZM fails to support these hypotheses. When comparing the results of the current study to that of the Bowen and coauthors (2022) study, a larger relative increase in AIAAD was observed when comparing treatments within each experiment. Therefore, it may be reasoned that increased PDT, along with 0% AZM inclusion may have influenced some anti-nutritional factors to ultimately increase AIAAD. Hence, the authors hypothesized, post-hoc, that the increased heat and shear forces lead to the deactivation of TI and CTI or aleurone layer degradation.

SDS-PAGE and immunoblot analysis confirmed the qualitative presence of TI and CTI in all samples (Kim and Krishnan, (2023); Figure 1). However, quantitative analysis of TIU and chymotrypsin inhibitor activity (**CIU**) of the soybean meal, mixed mash composites, and cooled pellet composites of the feed did not explain the increase in AIAAD seen in the 45mm control treatment (Table 6). When comparing the Maverick (69.16 TIU/mg and 12.42 CIU/mg) and Williams 82 (51.95 TIU/mg and 10.19 CIU/mg) raw soybean meal standards to the soybean meal used in the rations of this experiment (2.57 TIU/mg and 1.35 CIU/mg), it can be reasoned that the soybean meal was adequately heat-processed prior to pelleting and feeding. Further comparison of the experimental soybean meal to the average of the mixed mash composites (0.88 TIU/mg and 0.38 CIU/mg) shows a reasonable dilution of each inhibitor in the full diet. A final comparison of the 45mm control mixed mash (0.35 TIU/mg and 0.16 CIU/mg) to the cooled pellet composite of the same treatment (0.87 TIU/mg and 0.26 CIU/mg) provides no evidence to believe that deactivation of TI or CTI was the cause of the increased AIAAD. Therefore, the authors concluded that TI and CTI deactivation was likely not the driving factor in increasing AIAAD.

The authors finally speculate that the increased AIAAD observed in the 45mm control treatment was due to aleurone cell wall lysis caused by the added stress of increased PDT and no inclusion of AZM. The aleurone layer of corn is a consistently single-celled layer located between the pericarp and starch granules of the endosperm, therefore variation between the quality and quantity of its nutrient content is likely largely due to growing conditions (Singh et al., 2001; Ndolo et al., 2015). Geisler-Lee and Gallie (2005) propose that the aleurone layer of cereal grains (i.e., corn) synthesizes and contains little starch while containing relatively large quantities of protein. However, the cell walls of the aleurone layer are thick and constitute large proportions of cellulose and non-starch polysaccharides, much of which is indigestible to monogastric animals such as poultry (Moreau et al., 2001; Garcia-Lara et al., 2019). Therefore, exogenous enzymes or mechanical stress may be necessary to break through these cell walls. Saunders and coauthors (1969) saw a decrease in fecal protein content in broilers fed pelleted wheat bran (7.41%) when compared to the mash feed (8.27%), thus indicating more protein was digested and absorbed ($P < 0.05$). The authors of that experiment further analyzed the fecal contents and recognized an increased amount of broken aleurone layer cell walls of the pelleted wheat bran treatment compared to the mash and autoclaved mash samples ($P < 0.05$). Therefore, they attributed the increased protein utilization to the increased pressure and shear forces associated with pelleting increasing the accessibility of the protein contents of the aleurone layer cells (Saunders et al., 1969).

While the particle size of the crumbled feed presented to the broilers of this experiment likely did not confound the performance metrics, the particle size of the mixed mash, prior to pelleting, could have influenced aleurone cell wall degradation (Table 3). The mean particle size of the mixed mash samples taken prior to pelleting was 1093.866 µm, indicating that the average particle size of corn produced in this study was potentially large enough to increase the amount of shear forces between the pellet die and feed. Therefore, it may be reasoned that added mechanical stresses (heat, pressure, and shear) caused by the 45mm PDT compared to the 32mm PDT in combination with 0% AZM inclusion could have led to deformation and breaking of the aleurone cell walls which, in turn, allowed enzymatic access into the protein-rich interior, thus increasing AIAAD. Conversely, AZM inclusion potentially decreased the amount of friction between the feed and pellet die through the proposed mechanisms of pellet die scouring and lubrication, consequently sparing the aleurone layer cells from mechanical degradation (Knarr et al, 2023).

In conclusion, the authors recognize that the AIAAD increase seen for the 45mm control treatment may be due to variations in corn nutrient quality, the particle size of the mash feed pelleted, as well as the proposed added stresses caused by the 45mm PDT when compared to past research by Bowen and coauthors (2022).

CONCLUSIONS AND APPLICATIONS

1. Increasing PDT, in the absence of AZM, may increase AIAAD due to aleurone cell wall lysis. However, the marginal one to five percentage point increase in AIAAD was likely not large enough to increase broiler performance.

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TABLES AND FIGURES

Table 1. Diet compositions and calculated nutrient values of the Control and Azomite treatments reformulated for feeding in the starter phase.

Ingredient, %	Control	Azomite
Corn	51.25	51.01
Soybean meal (45.7%)	31.32	31.32
DDGS	4.75	4.75
Soybean Oil	4.76	4.76
Dicalcium Phosphate	2.01	2.01
Limestone	1.1	1.1
Salt	0.12	0.12
L-Methionine	0.23	0.23
Vitamin/Mineral Premix[1]	0.09	0.09
L-Lysine	0.24	0.24
L-Threonine (80)	0.04	0.04
Sodium Bicarbonate	0.09	0.09
Azomite	0	0.24
Calculated Nutrient Values, %		
ME (kcal/lbs)	1361	1361
Crude Protein	20.21	20.21
Digestible Lysine	1.02	1.02
Digestible TSAA	0.79	0.79
Digestible Methionine	0.52	0.52
Digestible Threonine	0.68	0.68
Digestible Tryptophan	0.23	0.23
Calcium	0.96	0.96
Available Phosphorus	0.48	0.48
Sodium	0.17	0.17

[1] 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. Lab analyzed nutrient concentration of the Control and Azomite diets and the major ingredients utilized

Diet\Nurient[1]	Crude Protein[2]	Crude Fat[3]	Crude Fiber[4]	Ash[5]	Ca[6]	P[7]	Moisture[10]
32mm Control	20.44	7.43	2.50	6.19	1.11	0.817	10.69
32mm + 0.25% AZM	20.78	7.44	2.64	6.08	0.994	0.747	10.37
45mm Control	20.98	7.51	2.56	5.60	0.920	0.670	9.96
45mm + 0.25% AZM	19.95	7.57	2.64	6.34	1.15	0.759	10.33
Corn	10.595	2.85	1.57	1.095	-	-	13.88
Soybean Meal	45.76	1.325	3.515	6.265	-	-	11.665
DDGS	30.55	4.63	8.54	5.03	-	-	12.41

[1] All nutrient results are presented as percentages of the total nutrient profile

[2] Crude protein was determined using the LECO method (AOAC Official 992.23/990.03)

[3] Crude fat was determined using petroleum ether in a Soxhlet apparatus method (AOAC Official Method 920.39).

[4] Crude fiber was determined by extracting fiber residue to be weighed, then ignited and reweighed to calculate the loss on ignition of residue (AOAC Official Method 973.18).

[5] Ash was determined by heating the sample for 2 h at 600°C (AOCS Official Method Ba 5b-68).

[6] Total calcium was determined by atomic absorption spectroscopy (AOAC Method 968.08).

[7] Total phosphorus was determined by colorimetric spectroscopy (AOAC Method 965.17).

[10] Moisture was determined by the freeze-drying method (AOAC Official Method 934.01).

Table 3. The average particle size of mixed mash and reground feed[1]

Diet	Mixed Mash[2]	Reground Feed[3]
32mm Control	1065.143 (1.990)	1285.132 (1.994)
32mm + 0.25% AZM	1103.028 (1.989)	1341.322 (1.955)
45mm Control	1104.015 (1.987)	1475.649 (2.046)
45mm + 0.25% AZM	1103.279 (1.980)	1472.720 (2.047)

[1] Values as presented: "Geometric Mean Diameter (Standard Deviation of Sample)"

[2] Mixed Mash samples originated from the diet prior to pelleting in Knarr and coauthors (2023)

[3] Reground Feed samples originated from the feed fed to the broilers of this experiment

Table 4. The performance response of Ross 308 based on AZM inclusion rate and PDT from day 0 to 21

Dietary Treatments	D 0-21 FI per Pen (kg)[1]	D 0-21 FI per Bird (kg)	D 0-21 LWG per Bird (kg)[2]	D 0-21 Bird Weight (kg)	D 0-21 FCR (kg/kg)[3]	Mortality Percentage
32mm Control	9.893	0.989	0.880	0.845	1.222	0.000
32mm + 0.25% AZM	10.172	1.017	0.908	0.873	1.214	0.000
45mm Control	9.902	1.012	0.898	0.862	1.211	2.000
45mm + 0.25% AZM	9.990	1.009	0.891	0.855	1.230	1.000
Treatment SEM[4]	0.140	0.015	0.012	0.012	0.011	1.134
Treatment P-value	0.472	0.582	0.443	0.448	0.599	0.532
LSD Value	-	-	-	-	-	-
Azomite						
0%	9.897	1.001	0.889	0.854	1.217	1.000
0.25%	10.081	1.013	0.900	0.864	1.222	0.500
Azomite SEM	0.099	0.011	0.009	0.009	0.008	0.802
Azomite P-value	0.199	0.408	0.412	0.405	0.609	0.663
Azomite LSD Value	-	-	-	-	-	-
Die Size						
32mm	10.033	1.003	0.894	0.859	1.218	0.000
45mm	9.947	1.011	0.894	0.859	1.221	1.500
Die Size SEM	0.099	0.011	0.009	0.009	0.008	0.802
Die Size P-value	0.538	0.622	0.995	0.966	0.795	0.197
Die Size LSD Value	-	-	-	-	-	-
Interaction SEM	0.140	0.015	0.012	0.012	0.011	1.134
Interaction P-value	0.500	0.319	0.161	0.167	0.222	0.663
32mm Control vs. 32mm + 0.25% AZM						
Control	9.893	0.989	0.880	0.845	1.222	0.000
0.25% AZM	10.172	1.017	0.908	0.873	1.214	0.000
SEM	0.133	0.013	0.015	0.015	0.014	0.000
P-value	0.171	0.171	0.207	0.209	0.698	-
LSD Value	-	-	-	-	-	-
45mm Control vs. 45mm + 0.25% AZM						
Control	9.901	1.012	0.898	0.862	1.211	2.000
0.25% AZM	9.990	1.009	0.891	0.855	1.230	1.000
SEM	0.118	0.017	0.010	0.010	0.011	1.650
P-value	0.611	0.914	0.609	0.620	0.244	0.678
LSD Value	-	-	-	-	-	-

[1] Feed Intake
[2] Live Weight Gain
[3] Mortality corrected Feed Conversion Ratio
[4] Standard error of the mean
[a-c] Means within a column not sharing a common superscript differ significantly ($P < 0.05$)

Table 5. Apparent ileal amino acid digestibility based on AZM inclusion rate and PDT found utilizing Ross 308 broilers

Dietary Treatments	Alanine	Aspartic Acid	Cysteine	Glutamic Acid	Glycine	Isoleucine	Leucine	Lysine	Methionine	Proline	Threonine	Valine
32mm Control	78.951^{b}	77.758^{b}	65.828^{b}	85.356^{b}	73.258^{b}	79.206^{b}	82.833^{b}	83.305^{b}	90.574^{b}	82.565^{b}	74.913^{b}	77.174^{b}
32mm + 0.25% AZM	79.651^{b}	78.602^{b}	66.118^{b}	86.036^{b}	73.994^{b}	80.081^{b}	83.667^{b}	84.267^{b}	91.442^{ab}	82.642^{b}	75.270^{b}	78.175^{b}
45mm Control	82.719^{a}	82.135^{a}	71.500^{a}	88.371^{a}	78.551^{a}	83.372^{a}	86.420^{a}	86.460^{a}	92.546^{a}	86.052^{a}	79.782^{a}	81.771^{a}
45mm + 0.25% AZM	79.187^{b}	78.319^{b}	66.915^{b}	85.836^{b}	73.994^{b}	79.206^{b}	83.404^{b}	84.087^{b}	91.3770^{ab}	83.203^{b}	75.409^{b}	77.759^{b}
Treatment SEM[1]												
Treatment P-Value	0.0125	0.003	0.0462	0.0028	0.0039	0.0076	0.0067	0.0199	0.0379	0.004	0.0036	0.0081
LSD Value	2.434	2.366	4.407	1.592	2.970	2.474	2.066	1.994	1.308	2.018	2.787	2.741
Azomite												
0%	80.835	79.947	68.664	86.864	75.905	81.289	84.627	84.883	91.560	84.309	77.348^{a}	79.473
0.25%	79.419	78.461	66.517	85.936	73.969	79.896	83.536	84.177	91.410	82.923	75.340^{b}	77.967
Azomite SEM												
Azomite P-Value	0.1029	0.0795	0.1687	0.1025	0.0694	0.1139	0.137	0.3136	0.741	0.0565	0.0461	0.1227
Azomite LSD Value	-	-	-	-	-	-	-	-	-	-	1.971	-
Die Size												
32mm	79.301	78.180^{b}	65.973^{b}	85.696^{b}	73.601^{b}	79.644^{b}	83.250^{b}	83.786^{b}	91.008^{b}	82.604^{b}	75.092^{b}	77.675^{b}
45mm	80.953	80.227^{a}	69.208^{a}	87.104^{a}	76.273^{a}	81.542^{a}	84.912^{a}	85.274^{a}	91.962^{a}	84.628^{a}	77.596^{a}	79.765^{a}
Die Size SEM												
Die Size P-Value	0.0592	0.0183	0.0424	0.0162	0.0146	0.0345	0.0272	0.0394	0.0438	0.0072	0.0147	0.0356
Die Size LSD	-	1.673	3.116	1.126	2.100	1.749	1.461	1.410	0.925	1.427	1.971	1.938
Interaction SEM												
Interaction P-Value	0.0178	0.0081	0.1201	0.0068	0.0163	0.013	0.0117	0.0222	0.0321	0.0449	0.0205	0.0132

[1] Standard error of the mean

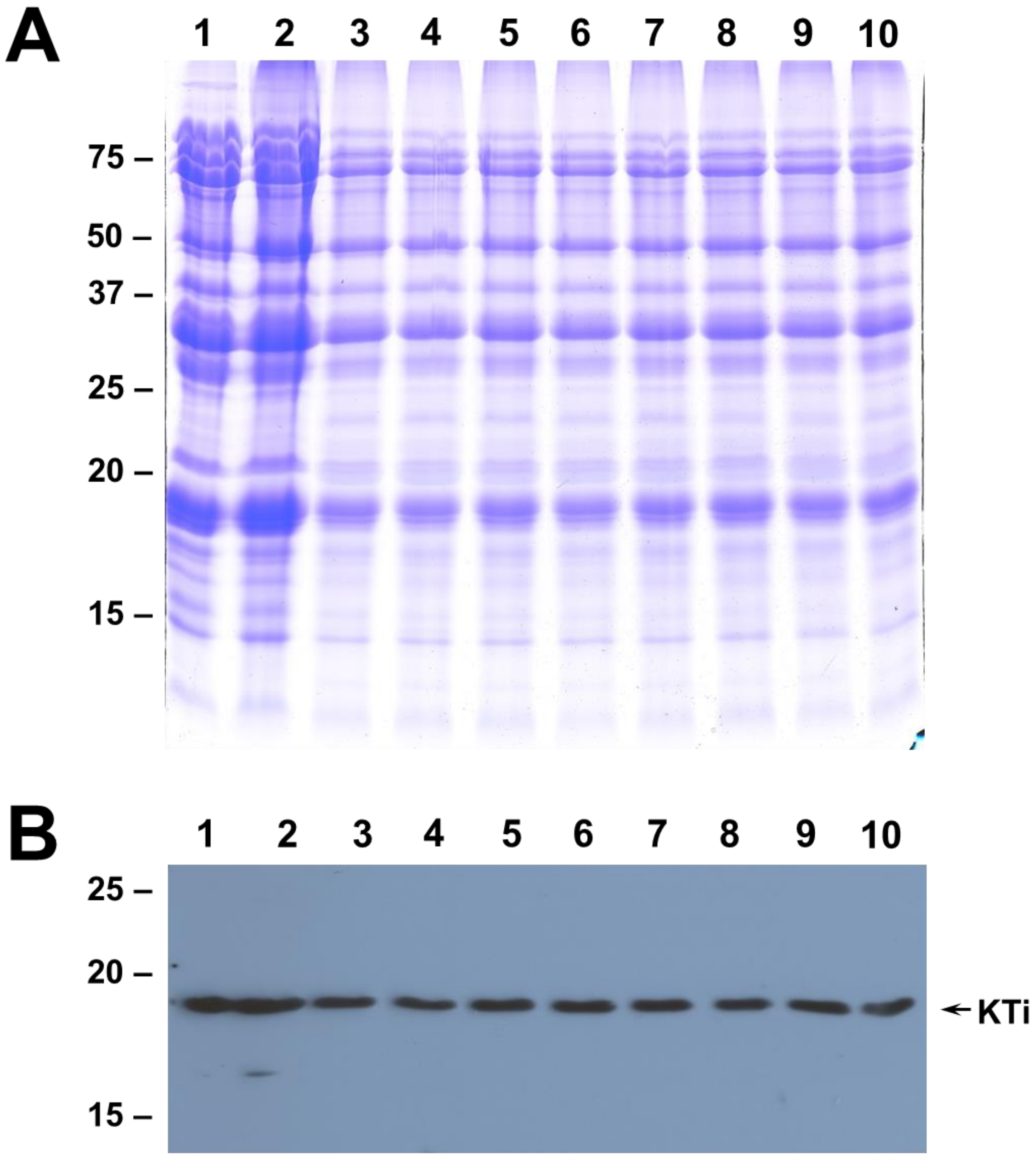
$^{a-b}$ Means within a column not sharing a common superscript differ significantly ($P < 0.05$)

Table 6. Trypsin and chymotrypsin inhibitor concentrations of various standards, ingredients, and treatments

Sample Description	TIU/mg of SBM[4]	CIU/mg of SBM[5]
Maverick RSBP[1,2]	69.16 ± 7.23	12.42 ± 0.41
Williams 82 RSBP[2]	51.95 ± 5.52	10.19 ± 0.27
Soybean Meal[3]	2.57± 0.39	1.35 ± 0.01
32mm Control Mash	1.03 ± 0.13	0.47 ± 0.12
32mm Control Pellet	0.83 ± 0.14	0.29 ± 0.04
32mm + 0.25% AZM Mash	0.81 ± 0.13	0.52 ± 0.05
32mm + 0.25% AZM Pellet	0.91 ± 0.12	0.35 ± 0.07
45mm Control Mash	0.35 ± 0.05	0.16 ± 0.07
45mm Control Pellet	0.87 ± 0.13	0.26 ± 0.01
45mm + 0.25% AZM Mash	1.32 ± 0.15	0.38 ± 0.05
45mm + 0.25% AZM Pellet	0.64 ± 0.10	0.33 ± 0.00

[1] RSBP: Raw Soybean Powder
[2] A standard used for comparison
[3] The soybean meal utilized to manufacture the experimental feed
[4] Trypsin Inhibitor Units
[5] Chymotrypsin Inhibitor Units

Figure 1. SDS-Page gel electrophoresis and chemiluminescent detection of Trypsin inhibitor in a soybean meal standard, soybean meal utilized in formulation, mixed mash, and cooled pellet samples of each treatment.



Lane 1: Maverick RSBP (Standard)
Lane 2: Soybean meal
Lane 3: MMC for 32mm Control
Lane 4: MMC for 32mm + 0.25% AZM
Lane 5: MMC for 45mm Control
Lane 6: MMC for 45mm + 0.25% AZM
Lane 7: CPC for 32mm Control
Lane 8: CPC for 32mm + 0.25 AZM
Lane 9: CPC for 45mm Control
Lane 10: CPC for 45mm + 0.25% AZM

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Education

Master of Science in Nutritional and Food Science — Expected Graduation 12/2023
West Virginia University, Morgantown, WV
Current GPA: 4.0

Bachelor of Science in Animal and Nutritional Sciences — Graduation 5/2022
West Virginia University, Morgantown, WV
GPA: 3.92 (Summa Cum Laude)

Achievements

- Eagle Scout Award (2019)

Teaching Experience

- Teaching Assistant for:
  - ANPR 367 (Poultry Production)
    - Gave lectures on basic feed manufacture and nutrition principles
  - ANPR 369 (Poultry Production Lab)
    - Assisted Undergraduate students with obtaining, rearing, and processing broilers
  - ANNU 362 (Applied Animal Nutrition II)
    - Gave Lectures on water, carbohydrates, protein, digestive physiology, feed manufacture, and diet formulation.

Oral Presentations

- International Poultry Scientific Forum 2023 (Atlanta, GA)
  - Knarr, L.E., K.M. Bowen, J. Ferrel, and J.S. Moritz. The effect of a *dacitic* (*rhyolitic*) *tuff breccia* (*Azomite*®) in corn, soybean, and DDGS-based diets across pellet die size on production rate and pellet quality under a constant motor load.
- Northeast President and Administrator Conference for the Northeast Region Farm Bureau States 2023 (Shepherdstown, WV)
  - Knarr, L.E., K.M. Bowen, J. Ferrel, and J.S. Moritz. The effect of a *dacitic* (*rhyolitic*) *tuff breccia* (*Azomite*®) in corn, soybean, and DDGS-based diets across pellet die size on production rate and pellet quality under a constant motor load.

Poster Presentations

- North American Biochar & Bioenergy Conference 2022 (Morgantown, WV)
  - Knarr, L.E., S.T. Grushecky, and J.S. Moritz. Biochar use in ammonia emission reduction, moisture content, and composting of chicken broiler litter.
- Graduate Student Research and Creative Scholarship Day 2023 (Morgantown, WV)

    - Knarr, L.E., K.M. Bowen, J. Ferrel, and J.S. Moritz. The effect of a *dacitic* (*rhyolitic*) *tuff breccia* (*Azomite*®) in corn, soybean, and DDGS-based diets across pellet die size on production rate and pellet quality under a constant motor load.

Publications

- 2023
    - L.E. Knarr, K.M. Bowen, J. Ferrel, and J.S. Moritz. 2023. Azomite®, a *Dacitic (Rhyolitic) Tuff Breccia*, included at 0.25% in Feed Manufactured with 32, 38, and 45mm Pellet Die Thicknesses Increased Pellet Production Rate by 5.0, 7.9, and 11.8%, Respectively. J. Appl. Poult. Res. **Submitted for Revisions**

West Virginia State Extension

- Poultry Workshop (Rearing and Processing)
    - Roan County (4-26-22)
    - Barbour County (5-12-22)
    - Reedsville, WV (5-16-22)
    - Wheeling, WV (6-3-22)
    - Parkersburg, WV (8-10-22)
    - Parkersburg, WV (4-21-23)
- Poultry Presentation (Rearing)
    - Wyoming County (6-7-22)
    - Glenville, WV (3-21-23)
    - Morgantown, WV (7-13-22)
- Poultry Processing
    - Barbour County (5-10-22)
    - Morgantown, WV (7-14-23)
- Judging
    - FFA Poultry CDE in Morgantown, WV (9-21-22)
    - FFA Egg Judging in Tyler County (3-8-23)
    - 4H Poultry Judging in Moorefield, WV (7-19-23)
    - FFA Poultry CDE in Morgantown, WV (10-05-23)
    -

Work Experience

Feed Mill Manager and Graduate Research Assistant, 05-2022 – Current
West Virginia University, Morgantown, WV

- Responsible for routine cleaning and maintenance of WVU Pilot Feed Mill and associated warehouse
- Responsible for ingredient apprehension and storage
- Responsible for the start-up and partial operation of the Feed Mill
- Worked with lab mates, farm workers, and my graduate advisor to plan and carry out feed manufacture for research and non-research animals
- Trained students on various tasks necessary for Feed Mill operation

Feed Mill Operator and Delivery Driver, 06-2018 – 08-2021
Haag's Feed Store and Milling, Troutville, PA

- Responsible for partial operation of Feed Mill manufacturing feed for all classes of production and show livestock and poultry
- Responsible for order apprehension and delivery for in-store and out-of-store purchases
- Responsible for inventory management and rotation of products
- Assisted with ration formulation and consultations with customers

Awards

Presentations

- Certificate of Excellence in recognition of an outstanding graduate student research presentation at the annual meeting of the Southern Poultry Science Society (2023)