ABSTRACT

RUBIO, ANDREA AZUCENA. The effects of Mixing and Pelleting Technological Applications on Feed Quality Parameters and Broiler Growth Performance (Under the direction of Dr. Adam C. Fahrenholz and Dr. Jesse Grimes).

A series of experiments were conducted to determine the impact of mixing parameters and in-line mixing and pelleting technologies to improve current feed manufacturing techniques and efficiency in the process flow. The first experiment examined the effects of two mix times on coefficient of variation (CV), individual body weight (BW) uniformity, and broiler growth performance from 1 to 42 d of age. Batches of feed were mixed for 4.5 min (3 min dry mix and 90 s of wet mix) and 30 s (0 s dry mix and 30 s wet mix) to obtain a Uniform (UM) and a Non-uniform (NUM) mix, respectively. Each pen was randomly assigned to 1 of 4 dietary treatments: 1) UM from 1-42 d, 2) UM from 1-28 d and NUM from 28-42 d, 3) UM from 1-14 d and NUM from 14-42 d, and 4) NUM from 1-42 d. Ten samples were collected at equally spaced time intervals from the mixer discharge conveyor after each mixing period and analyzed for CV using multiple tracers. The experimental design consisted of a 1-way treatment structure using a randomized complete block design with pen location being the blocking factor. There were no statistical differences between the treatments on BW, feed intake, feed conversion ratio (FCR), and BW uniformity during the starter, grower, and finisher periods ($P > 0.05$).

A second experiment was conducted to evaluate the interactive the effects of mix time, batch size, marker selection, and In-line near infrared (NIR) spectroscopy on mixer CV. Twelve batches of feed were mixed with 3 replicate batches/treatment. The batches of feed were mixed for 4.5 and 0.5 min with the same dry and wet cycles of experiment 1. The experiment constituted a 2×2×4 factorial arrangement of two mix times, (4.5 and 0.5 min), two batch sizes (1 and 2 Tons), and four methodologies to evaluate mixer performance for CV determination

(sodium chloride, Microtracers (Red and Blue #40), and In-line NIR). For main effects, there were no statistical differences between batch sizes on CV ($P > 0.05$). However, a mix time of 4.5 min and the In-line NIR generated CVs less than 10% compared to 0.5 min, sodium chloride, and the Microtracers. Interactions were apparent ($P < 0.05$) only for methodology and total mix time.

A third experiment was conducted to evaluate the current available non-contact methodologies compared to a novel in-line methodology to estimate the rise in hot pellet temperature (HPT) as they are extruded by the die. In experiments 1 and 2, three non-contact methodologies were used to collect 189 HPT readings at die exit: 1) Insulated thermos, 2) Styrofoam bucket, and 3) infrared (IR) gun. Experiment 3 constituted a 2×2×3 factorial arrangement of 2 fat inclusion levels (1 and 4%), 2 die L:D ratios (6.5 and 10), and 3 conditioning temperatures (74°, 79°, and 85° C). A temperature profile probe with 8 sensing points located within the pelleting chamber was used to collect HPT readings at the die surface in real time. In experiment 1 and 2, the lowest HPT was obtained when the sample was analyzed with the IR gun ($P < 0.01$). The temperature readings obtained with the IR gun in these studies fluctuated at ambient temperature which allowed heat to escape within the pellets, leading to an underestimation of HPT. In experiment 2, interactions were apparent ($P < 0.01$) for all treatments. Diets pelleted with a thicker die, 1% fat level and conditioned to 85° C had the highest HPT readings ($P < 0.01$). The results of these experiments indicated that HPT readings differed depending on the methodology used.

The effects of Mixing and Pelleting Technological Applications on Feed Quality Parameters and Broiler Growth Performance

by
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DEDICATION

This dissertation is dedicated to the people who have supported me throughout my education.

BIOGRAPHY

Andrea Azucena Rubio Molina was born on September 25th, 1993 to Mabil Molina and Maynor Rubio in San Pedro Sula, Honduras. She grew up in San Pedro Sula, Cortes. She graduated from Saint Peter's Academy High School in San Pedro Sula, Cortes in 2011 and attended Zamorano University in 2012 to study agronomy. Andrea worked as a broiler management intern at Louisiana State University and graduated with her Bachelor of Science from Zamorano in December of 2015. In 2016, Andrea attended Auburn University to pursue a Master of Science in Poultry Science under the direction of Dr. Wilmer Pacheco. After completion of her Master of Science, Andrea transferred to NC State to fulfill the doctoral requirements at NC State in Animal and Poultry Science and Nutrition under the direction of Dr. Adam Fahrenholz.

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LITERATURE REVIEW

IMPORTANCE OF THE MIXING PROCESS IN THE BROILER FEED INDUSTRY

Feed costs can represent up to 70% of the total broiler production costs (Behnke and Beyer, 2002). Therefore, feeding programs for broilers are designed to meet their nutritional needs at the lowest possible cost while maintaining efficiency in the feed manufacturing process (Dozier et al., 2006). Therefore, any improvement in feed manufacturing efficiency will have a tremendous impact on the bottom line. In every feed manufacturing facility, the mixing process is a key component to achieve a complete nutrient ration regardless of the feed form (mash, crumbles, or pellets) being fed to the animal. Broilers consume less amount of feed in each bite compared to swine and cattle hence a proper mixing time and nutrient homogeneity are required to maximize nutrient consumption and optimize their growth performance (Creger, 1957; McCoy et al., 1994). Therefore, mixer performance is an important quality control point in broiler feeds. Previous research has reported that a good mix or a uniformly mixed diet guarantees the optimum nutrient levels and delivers the majority of essential nutrients needed for growth performance and health thus meeting manufacturing standards set by nutritionists and feed manufacturers (Creger, 1957; Martin. 1985; Beumer. 1991; Wicker and Poole, 1991; McCoy et al., 1994). Moreover, mixing uniformity is influenced by mix time and mixer performance. Recent studies evaluating the influence of mixing uniformity in broiler diets have produced inconsistent results in relation to growth performance, defining an adequate mix time, marker selection, and equipment used (Ciftci and Ercan, 2003; Clark et al., 2007; Reese et al., 2017). These studies suggest that the mix uniformity fed in poultry diets has an impact on growth responses and economical revenues in the modern commercial broiler industry.

Mixer performance analysis

Testing mixer performance is essential to achieve nutrient homogeneity, optimum growth performance, regulatory compliance, and the optimization of the mixing process and equipment. Pfost et al. (1974) reported three causes of nutrient variation: 1) nutrient variation of ingredients between batches, 2) batching errors, and 3) poor mixing or nutrient segregation after mixing, hence a methodology to test mixer performance was developed. Creger (1957) reported that a minimum of 9 samples from a batch of feed should be collected and analyzed to obtain an accurate representation of the uniformity of mixed feed. Therefore, the current methodology to test mixing uniformity is determined by selecting a marker (e. g. minerals) or ingredient (e. g. salt or sodium) in the diet formulation and calculating its distribution or coefficient of variation (**CV**) in a set number of samples (10) across a batch of feed (Reese et al., 2017). Pfost et al. 1966a reported that a CV of 20% may cause toxicity problems with some medications in feed. Regardless of the aforementioned causes, the industry has accepted a CV of 10% as a standard for any mixing process assay in which a CV $\leq$ 10% represents a uniform mixed diet (Pfost et al., 1966a; Beumer, 1991). Therefore, mix uniformity is a quality parameter that can be controlled or monitored depending on the time that ingredients spend in the mixing chamber and/or the marker selected to test mixer performance. Previous research has reported that mixing uniformity is indirectly proportional to mixer capacity and mixing time (Wicker and Poole 1991; McCoy et al., 1994). Ideally, longer mix times and optimum batch size should report a CV $\leq$ to 10%. However, the interpretation of a mixer CV analysis may vary depending on the marker selected, inclusion rate and/or particle size of a marker, mixer type, and mixer CV assay used (Herrman and Behnke, 1994; McCoy et al., 1994; Groesbeck et al., 2007; Fahrenholz and Stark, 2014). A high mixer CV result could be used to correct improper mixing or monitor mixer performance.

Factors influencing mix uniformity

Understanding the impact of mixing systems on nutrient homogeneity allows a feed mill manager or nutritionist to optimize time management within the feed facility and broiler growth. The objective of modern batching systems is to accurately convey each ingredient of the diet formulation into the mixer at the lowest possible cost. Once ingredients have been properly weighed, the batching sequence begins and continues until discharge of the mixer. This is a critical point of the mixing process because factors within the equipment such as mixer type, marker, or the predetermined batching sequence have an impact on the efficiency of the mixer and the CV of the assay.

Equipment. The design of the mixer and the different mixing zones within a chamber may have an impact on mix uniformity. Horizontal mixers are commonly used in vertical integrated (broilers) feed mills compared to smaller facilities (cattle) which use vertical mixers because they work well for high-forage rations and have lower maintenance costs (Stark and Saensukjaroenphon, 2017). Marczuk et al. (2017) reported that horizontal mixers have the ability to uniformly blend ingredients with different particle size and bulk density in the least amount of time possible. The versatility and design factors of horizontal mixers makes them well suited for the broiler feed industry. Previous research has reported that increasing wet mix time did not affected mixer CV on a twin shaft counterpoise mixer but had the opposite effect on a paddle and double ribbon mixer (Saensukjaroenphon et al., 2019). The authors concluded that the amount of time required to add liquid ingredients into the diet and wet mix time depend on mixer type and size. In agreement, Stark and Saensukjaroenphon (2017) reported that double or twin shaft mixers achieve a rapid blending while creating a uniform dispersion of the ingredients in the diet. These studies suggest that differences in mixing zones and variety of configurations

within the mixing chamber have an impact on mix uniformity and predetermination of the batching sequence.

Batch size. Feed manufacturers recommend maintaining an even fill of the mixer to guarantee a complete blend of ingredients. However, previous research has reported that overfilling of the mixer causes the feed particles to float outside the mixing zones increasing mixer CV regardless of mix time (Wicker and Poole, 1991). The authors concluded that mixing an additional 907 kg batch over mixer capacity, decreases mix uniformity regardless of mix time or even if mix time is increased. In contrast, underfilling of the mixer may not provide enough contact between the assemblies and free-flowing ingredients which can have a negative impact on mixer performance (Martin, 2005).

Marker. A marker is a nutrient or ingredient used to calculate the CV of a diet and is part of quality assurance program. Marker selection is essential to obtain an accurate %CV and avoid overestimation or underestimation of mix uniformity. Previous research recognizes the characteristics of an adequate marker for testing mixer performance: single source ingredient, ingredients with similar particle size and density, accurate analytical assays, variability of on-site vs. laboratory assays, and the assay should not be cost prohibitive (Pfost et al., 1966b; Martin, 2005; Clark et al., 2007). Like previously mentioned, a longer mix time should increase mix uniformity thus report a CV $\leq$ 10%. Clark et al. (2007) reported that even though manganese, microtracers (Fe colored particles), and semduramicin resulted in decreased CVs when mix time increased from 0.5 min to 5.0 min, all the calculated CVs of these markers remained > 10%. In contrast, DL-Methionine and L-Lysine-HCl were the only markers that followed an adequate trend for estimating mix uniformity. McCoy et al. (1994) reported that the %CV for sodium chloride concentration decreased from 43% to 10.8% and 13.1% with mixer revolutions of 20,

40, and 80, respectively. In contrast, in the study by Clark et al. (2007), there were no statistical differences in %CV for sodium chloride at 0.5 min, 2.5 min, and 5.0 min of total mix time. The authors concluded that even though sodium chloride is considered a single source, the chloride analysis or titration method used (adopted by the industry) may interfere with the results when other ingredients with the chloride ion are included in the diet. Both of these studies used a double-ribbon mixer, nevertheless differences in batch size (86 kg vs. 454 kg), sample size (0.2 kg vs. 5 kg) and sampling location (10 random locations throughout the mixer vs. sacks) are an indication that each analysis is unique to a mixing system which may impact mix uniformity results and interpretations of a "good" and/or "poor" CV.

EFFECTS OF MIX UNIFORMITY ON BROILER GROWTH PERFORMANCE

The evaluation of mix uniformity is crucial to revisit the main factors affecting mixer performance in modern equipment, level of tolerance in modern strains and impact on their growth performance, nutrient homogeneity, marker selection, and methodologies to calculate CV. Previous research has reported that a reduction in %CV thus increased mix time improves growth performance of swine and poultry (Duncan, 1989; Traylor et al., 1994). McCoy et al. (1994) reported that the average daily gain (**ADG**) and feed conversion ratio (**FCR**) improved when 28 d broilers were fed diets with increased mixing revolutions from 5 to 20 but no further improvements from 20 to 80. In a more recent study, birds fed diets mixed at 0.20 min, 0.59 min, and 3.75 min did not have an impact on body weight gain (**BWG**), feed intake (**FI**), FCR, and mortality at 42 d of age (Ciftci and Ercan 2003). In contrast, Rocha et al. (2022) reported that broilers fed diets mixed at 0.5 min and with a CV of 39.5% had lower BWG and FI from 1 to 40 d of age. However, no statistical differences in individual BW uniformity were observed. Swine and poultry have different feeding phases and nutrient requirements which have an impact on the

predetermination of batching sequences and mix time. Traylor et al. (1994) reported that nursery pigs were more susceptible to diet uniformity than finishing pigs. However, the authors suggested that although finishing pigs tolerated a diet with a CV up to 54%, the conveying systems of their facility likely decreased the CV. Thus, the driving factor of mix uniformity and growth performance is the CV of the diet and not the total mix time. A good example would be to consider a smaller mixing system with 0 min mix time. This increases the CV of the diet and has a negative impact on growth performance (Traylor et al., 1994). Furthermore, previous research has reported that when there is a deficiency in nutrient requirements of broiler diets, it becomes critical to decrease %CV as an alternative to improve growth performance (McCoy et al. 1994; Ciftci and Ercan, 2003). Currently, broiler diets are formulated to meet or exceed nutrient requirements. Hence, birds are able to consume diets with higher CVs, assuming adequate nutrient levels are met (Ciftci and Ercan 2003). Mix time has been reported as a major driving factor of mix uniformity (Duncan 1989; Traylor et al., 1994; Rocha et al., 2022). However, conflicting results among studies suggest that other factors such as the productive phase of broilers has an impact on mix uniformity requirements, thereby affecting growth performance and individual BW uniformity.

APPLICATION OF NEAR INFRARED (NIR) SPECTROSCOPY TECHNOLOGY TO ANIMAL FEED MANUFACTURING

Near Infrared (**NIR**) spectroscopy technology supports feed quality control through advancements in diet formulation hence is becoming increasingly more important in the poultry industry. The term “infrared” was first described in 1835 as a light with longer wavelength than visible light (Ozaki and Huck, 2007) and divided into three regions: NIR region (800-2,500 nm), the infrared region (**IR**) (2,500-25,000 nm), and the far-IR region (25 μm-1 mm) (Siesler

et al., 2008; Czarnecki et al., 2015). Although NIR spectroscopy remained as a concept rather than an application, it was not until the 1960s that an agricultural engineer Karl Norris found that grain had absorbances in the NIR region and started to develop calibrations from NIR data (Hart et al., 1962; Ozaki and Huck, 2007). Once the basic concepts of the NIR region were understood, the spectral analysis became part of NIR studies and its application to agriculture. The basic design of NIR analyzers consists of a light source, a monochromator that produces the spectrum, and a photodetector to recognize the wavelength (Huck, 2007). In other words, when the NIR resonates at a specific wavelength that correlates with molecular bonds (carbon, nitrogen, and hydrogen bonds) found in raw materials, the spectra is generated (Shenk et al., 2007). Then, the calibrations for these ground or un-ground samples are created and updated by using the prediction from previously calibrated samples and adjusting bias (Givens et al., 1997). Further advancements in NIR spectroscopy have allowed the development of computers, sensors, instruments, and spectrometers that can be used in many areas of animal and food science.

Currently, feed manufacturers and nutritionists use NIR equipment to predict the nutritional value of ingredients and feed samples. Previous research has reported that the monitoring and understanding of diet composition is essential for animal growth performance and to prevent diseases (Debry, 1992; Dijkstra, 1993). However, the chemical analyses of some feedstuffs can be cost prohibitive. Thus, NIR software and hardware developments offer non-contact analyses (Ozaki and Morizawa 2007), rapid results of newly and efficient laboratory-scale analyzers and sensors (Pierna et al., 2006), evaluation of chemical and physical characteristics of cereal grains (Owens et al., 2009), and have the ability to adapt to manufacturing settings as an in-line application (Graham et al., 2012). Therefore, the benchtop

NIR is mostly used by the animal feed industry to obtain a proximate analysis of a large number of samples and develop a database for decision-making during diet formulation (Park et al., 1998). Moreover, modern technologies allow the use of light-fiber probes and optical materials to be installed in industrial environments (e.g., feed conveying system) which makes NIR spectroscopy suitable for real time analysis at a feasible cost (Ozaki and Morizawa, 2007). Unlike raw materials, the nutritional value of animal diets varies as a result of ingredient composition, hence producing a large variation in spectra and physical and chemical characteristics (Givens et al., 1997; Rose et al., 2001). Therefore, the use of NIR equipment could be an alternative to improve feed efficiency by allowing nutritionists to monitor nutrient variation of ingredients and optimize diet formulation.

The advantages of NIR applications on broiler feeds

The nutrient variation within ingredients may have an impact on diet formulation and growth performance of broilers. Previous research has reported that genetic variety, harvesting season and conditions, and drying conditions have an impact on nutrient variation and digestibility of cereal grains (Kaczmareck et al., 2014; Yin et al., 2017; Nieto-Ortega et al., 2022). The proximate or nutrient content analyses obtained with the NIR can provide forewarning of possible problems taking place in the receiving process of ingredients or grain quality from different suppliers. Using NIR technology, Williams et al. (2018) reported variations of 4% and up to 25% in the content of starch, protein, fat, fiber, and moisture in corn, and statistical differences were observed in BWG and feed efficiency of broilers. The wet chemistry methods used to create the NIR system's calibrations can be cost prohibitive and have high turnaround results; thus, NIR technology has been investigated by researchers for applications other than the classic proximate analysis of ingredient samples (Nieto-Ortega et al.,

2022). For example, recent studies have reported that the NIR analytical methods can be used to predict antinutritional factors from wet chemistry methods (Aureli et al., 2017; Olukosi and Bedford, 2019) and as an indirect method to estimate wheat hardness because spectral characteristics vary with particle size distribution (Amerah, 2008). Feed particles and raw materials are subjected to manufacturing processes that alter their physico-chemical characteristics and nutritional composition. In some regions of the US, Canada, Australia, and Europe, NIR equipment is installed at grain elevators of feed facilities to collect proximate analysis of cereal grains (Sandra et al., 2005). The previously mentioned abilities of NIR's computers and sensing probes to adapt to industrial environments make it an analytical tool for other processing conditions in feed manufacturing. In agreement, previous research has evaluated the chemical composition of feed and compacting behavior of pellets during mixing and pelleting processes, respectively (Norris and Barnes, 1976; Nathier-Dufour et al., 1992). These studies suggest that the spectral characteristics within broiler diets and ingredients have an impact on manufacturing efficiency and the physical quality of feed in the modern feed industry. Therefore, even though NIR technology has been around for many years, the potential applications for ingredient quality control and the animal feed manufacturing industry, in general, are promising and require further exploration.

OVERVIEW OF FEED PELLETING IN THE POULTRY INDUSTRY

Pelleting is a technology used by feed mill operators and nutritionists to feed broilers and turkeys to optimize feed intake and nutrient digestibility. The pelleting process consists of the agglomeration of feed mash particles with the addition of heat, moisture, and pressure (Fairfield et al., 2005). The beneficial effects of pellets over mash diets on BW, feed intake, and FCR of broilers have been reported and supported by previous studies (Proudfoot and Sefton, 1978;

Proudfoot and Hulan, 1982; Choi et al., 1986; McKinney and Teeter, 2004; Corzo et al., 2011; Rubio et al., 2020). Behnke and Beyer (2002) reported the beneficial effects of feeding pelleted diets: decreased feed wastage, nutrient agglomeration, and improved palatability. Researchers, nutritionists, and feed manufactures continually explore options to improve FCR of broilers while being more efficient with feed production thus reduce manufacturing costs. However, pelleting is a complex process, which means that there are other factors involved that are not under the control of the nutritionist or the feed mill manager. Considering the importance of feed pelleting in poultry production, it is imperative to understand the current manufacturing practices that influence pellet quality and alternatives to make the pelleting process of broiler diets more efficient.

In any feed facility, pellet quality is determined by sifting (remove fines), tumbling/shearing into each other, and weighing pellet samples thus estimating the pellet durability index (**PDI**) (Thomas and van der Poel, 1996). Even though pelleting is a common practice to feed meat-type poultry, pellet quality can be neglected in vertically integrated poultry feed mills since a high throughput makes more financial sense for their business model. In agreement, Corzo et al. (2011) stated that in order to increase throughput, some feed facilities change die specifications and steam-conditioning parameters to meet their production goals of the week. However, even if the primary goal of integrated feed mills is not a high pellet quality, previous research has shown the detrimental effects of pellet fines and poor pellet quality on feed conversion of broilers and turkeys (Proudfoot and Hulan, 1983; Salmon 1985; Scheideler, 1991). Moreover, it has been hypothesized that pellet hardness should be considered as a synergistic component of pellet quality that may be overlooked when only PDI is determined (Parsons et al., 2006; Abdollahi et al., 2013). This supports the idea that pellet quality is a critical concept in the

understanding of the inter-relationships of better growth performance and maintaining the physical integrity of feed. Nonetheless, it is important to remember that pellet quality is influenced by temperature, moisture, and die specifications for which there are not set manufacturing standards. Therefore, future research should focus on understanding the physicochemical factors affecting the pelleting process as pellets are the agglomerated nutrients required by the animal to digest and convert to meat accretion.

EFFECTS OF DIET FORMULATION AND MANUFACTURING TECHNIQUES ON PELLET QUALITY AND FEED THERMOSTABILITY

Pellet Quality

During the pelleting process, pellets are expose not only to chemical alterations but are pressed and extruded by the die, discharged into the cooler, and conveyed through feeding systems which may affect its physical integrity. Therefore, a high pellet quality is obtained when pellets remain mostly intact after cooling and transportation processes without breakage and increased amount of fines. Previous research has shown that the main factors affecting pellet quality include formulation (40%), particle size (20%), die (15%), steam conditioning (20%), and cooling (5%) (Behnke, 2001). Even though diet formulation has a large impact on pellet quality, recent studies have shown that the combined effects of formulation (23%), conditioning (33%), and a greater role of the pellet mill die (43%) could have a significant impact on pellet quality (Fahrenholz, 2012; Pope and Fahrenholz, 2020). For purposes of this chapter, only the following factors affecting pellet quality will be discussed in greater detail: diet formulation, mash conditioning, and the pellet mill die.

Diet Formulation. It is known that modifications in the inclusion rate of an ingredient in a poultry diet has an impact not only on pellet quality but the economics of feed manufacturing.

While poultry diets have a variety of cereal grains, minerals, vitamins, amino acids, fats, and oils, least-cost formulation and ingredient availability are driving forces in the final nutrient content fed to the animal (Fairfield et al., 2005; Loar II and Corzo, 2011). Therefore, it is imperative to understand the effect of each feedstuff material in the binding mechanisms of feed mash particles on pellet quality (Kniep, 1982; Fairfield et al., 2005). Poultry diets in the U.S. are mainly composed of cereal grains like corn and soybean meal (**SBM**) which are the main sources of starch and protein. Starch gelatinization and protein denaturation have been reported to promote the binding properties of mash particles by breaking hydrogen bonds to engage more water and increased gel formations, respectively (Hermannson, 1979; Mommer and Ballantyne, 1991). In contrast, other studies have shown that the exposure of these particles to steam and pressure at the conditioner, are not the only thermomechanical factors affecting the binding properties of pellets (Collison and Chilton, 1974; Wootton and Bamunuarachchi, 1979; Gilpin et al., 2002; Moritz et al., 2003; Svihus et al., 2004). Even though cereal grains are major components of poultry diets, it should be noted that other feedstuff components have interrelated effects with these major ingredients that could have an impact on pellet quality. The conflicting results of starch and protein on pellet quality in these studies have led to consideration of the impact of moisture. During the pelleting process, water can be added in the form of steam at the conditioner but is also included to some extent in each individual ingredient of the diet (Loar II and Corzo, 2011). The inclusion of additional water in ranges of 2-4% result in improved pellet quality (Fairchild and Greer, 1999; Buchanan and Moritz, 2009) which has been attributed to increased starch gelatinization in the presence of additional moisture (Moritz et al., 2001). However, past literature shows that starch gelatinization may not be the determinant factor of pellet quality. Therefore, corn and SBM may not exert the greatest influence on pellet quality

like previously stated. Other ingredients including fiber, by-products and water may be an example of these interactions although research evaluating their impact on pellet quality is sparse.

Based on the literature reviewed, fat is one of few feedstuff materials that have consistent results on pellet quality when added in high amounts in poultry diets. Previous research has shown that the inclusion of fat above 2% results in poor pellet quality and increased amount of fines; however, a higher throughput and decreased energy usage are observed as a result of die lubrication while running the pellet mill (Richardson and Day, 1976; Salmon, 1985). The previously mentioned role of water on pellet quality and the repelling relationship of fat and water could play a major role in the pellet mill die in addition to its role in the mixer and post-pellet application. It seems that other than diet formulation, the mechanical effects of the equipment and the different composition of ingredients affect the physical integrity of pelleted poultry feeds.

Mash Conditioning. The gelatinization of starch and changes in protein plasticization promote the adhesion of mash particles during the conditioning process (Behnke, 1994; Behnke, 2001; Muramatsu et al., 2015). The conditioning process is considered the first step in feed manufacturing where mash particles are exposed to manually or automated controlled heat treatment before entering the pellet mill die. However, mash conditioning consists not only of the addition of heat to the feed mash particles but also water, high pressure, ambient atmospheric conditions, and indirect heat as a result of zones adjacent to the heat source (Fairfield et al., 2005). During the pelleting process, settings such as conditioning temperature, retention time, and steam injection are monitored and controlled by mill operators, which can lead to modifications in pellet durability and bacteria proliferation; hence previous research focuses on

the relationship between conditioner parameters and pellet quality (Smallman, 1996; Van Immerseel et al., 2009). Previous research has reported that increasing steam-conditioning and conditioning temperatures by 13 °C and 11.3 °C increases PDI by 3.2% and 4%, respectively (Skoch et al., 1981; Cutlip et al., 2008). In the case of steam pressure, Cutlip et al. (2008) reported that increasing steam pressure had no effect on PDI. Considering the impact of conditioning temperature and pressure on PDI, Briggs and other concluded that an adequate amount of pressure (241 to 276 kPa) (Briggs et al., 1999; Maier and Briggs, 2000), and providing the right amount of water, in the mash compared to variations in conditioning temperatures, could have a higher impact on PDI (Abdollahi et al., 2013). Furthermore, increases in conditioner retention time from 3 s up to 240 s has been reported to be beneficial for PDI in ruminant and poultry diets (Briggs et al.,1999; Massuquetto et al., 2018; dos Santos et al., 2020; Soltani et al., 2020). Similar to other manufacturing processes, mash conditioning is a sophisticated system that can be affected by other factors that are typically related to ambient conditions. A good example is the moisture content and storage conditions of grains throughout the year (Fairfield et al., 2005). As the year proceeds, drier grains enter into storage and when the steam is not able to adequately penetrate mash particles, conditioning temperature or target moisture may not be met (Fairfield et al., 2005; Behnke and Gilpin, 2014). In terms of pellet quality, the conditioning process is definitely critical before the mash enters the pellet mill die. However, the role of the rolls and the die on extruded pellets should be considered as inter-related components of the conditioner and the overall pelleting process. Therefore, it seems only logical to consider and understand all the factors involved in these processes and the physico-chemical changes of mash particles during conditioning and pelleting.

Pellet Mill Die. The conditioner and pellet mill die are pieces of equipment that may alter the physical integrity of finished feed pellets; hence the influence of conditioning parameters on pellet quality is better understood than the die itself. Although the pellet mill die is the "extrusion area" of the pelleting process, previous research has recognized that the die has a more significant impact on production rate and pellet quality than initially thought (Behnke, 2001; Fahrenholz, 2012; Pope and Fahrenholz, 2020). Therefore, feed manufacturers operate and control pelleting throughput by applying the concept of "effective thickness", which is the working part of the die (the length of the hole where the pellet is created) and varies depending on the diameter of the pellet (Fairfield et al., 2005). Behnke (1990) reported that an increase in die thickness improves pellet durability and has been supported by recent studies (Buchanan et al., 2010; Wamsley and Moritz, 2013). In contrast, thicker dies have been reported to have a negative impact on heat-labile ingredients (Bayley et al., 1968; Plavnik et al., 1997). A high pellet quality is not an option for feed facilities with larger capacities since they need to achieve tonnage in a shorter period of time which means higher throughput using thinner dies. This limitation causes modifications in the conditioning process and residence time of pellets in the die which may alter the binding mechanisms of feed pellets (Loar II and Corzo, 2011). Regardless of the potential impact of the die in the binding mechanisms of feed pellets, research evaluating thermal and mechanical processing techniques inside the pellet mill die on pellet quality is sparse. The main reason is that during the pelleting process, the door of the die chamber remains closed, steam is generated from thermal processing, and the die rotational speed and aggressiveness makes the collection of feed pellets for further analysis laborious and unattainable. Therefore, the pellet mill die could be a driving force of finished feed quality within the pelleting process and deserves further exploration.

Feed thermostability

Thermal processing of ingredients is a common practice in the feed animal industry. A review of the literature indicates a general agreement that diet formulation and conditioning parameters have an impact on the physical integrity of a pellet. Even though there are differences in ingredients, manufacturing conditions, and equipment used, the common goals are to control bacteria and feed hygiene which have been correlated with higher pelleting temperatures (Veldman et al., 1995; Jones and Richardson, 2004). In agreement, Thomas and Van der Poel 1996 stated that high temperatures are also related to a high pellet quality. However, the impact of temperature on nutrient levels and the thermo-mechanical changes of extruded pellets are often misunderstood by the poultry industry. Despite the impact of mash conditioning on pellet quality, there is little published data examining the effects of the potential strenuous impact of heat treatments applied by the pellet mill die on thermos-sensitive ingredients.

Starch. Starch gelatinization is a good example of a modified structure that benefits from heat-induced changes. A starch granule is mainly composed of amylose and amylopectin located in the semi-crystalline and crystalline regions, respectively. Water and temperature addition play a significant role in the process of starch gelatinization. Eliasson and Gudmundsson (1996) reported that starch granules start to gelatinize at temperatures between 45° C and 90° C. In contrast, Svihus and Zimonja (2011) reported that cereals surrounded by a high-water environment, will have a shift in range of temperature gelatinization from 50° C to 70° C. Hence, when temperature is applied, the structure of starch changes from crystalline to an amorphous structure but gelatinization occurs when water is absorbed, and hydrogen bonds are disrupted (BeMiller, 2011). Although temperature is an aid on starch gelatinization, further addition of heat

may lead to irreversible changes in the structure and lead to disintegration of the starch granule, which interferes with cooking processes of food products (Svihus and Zimonja, 2011). Therefore, heat addition of starch granules is a complex process as it involves water and residence time to achieve the desired level of gelatinization. Furthermore, conflicting results have been reported on the effects of pelleting on starch digestibility of broiler diets (Ankrah et al., 1999; Moritz et al., 2002; Svihus, 2011). Based on published literature, there is an agreement that heat treatments have an impact on the structural changes of starch, however, the pelleting process may not be a driving factor on the level of starch gelatinization or sole predictor of pellet durability. Even though the pelleting process is responsible for only a small extent of starch gelatinization, previous research has concluded that most of the starch gelatinization occurs at the extrusion point of the pellet mill die compared to the conditioner (Stevens, 1987; Heffner and Pfost, 1973; Zimonja et al., 2008; Abdollahi et al., 2010). Abdollahi et al., 2010 reported that starch gelatinization increased at lower conditioning temperatures thus supporting the concept of frictional heat at the die. In agreement, a later study reported that starch gelatinization of mash diets conditioned at temperatures of 60° C and 75° C while mash diets conditioned at 20° C and further pelleted had the highest starch gelatinization content (Abdollahi et al., 2011). Starch gelatinization benefits from conditioning and pelleting processes compared to the potential negative effects on survivability and retention of other ingredients. However, it is necessary to further evaluate the effects of current high temperatures (e.g., 91° C) and manufacturing techniques on the extent of starch gelatinization and digestibility.

Protein. Protein sources fed in broiler diets may come from plant (e.g., soybean or wheat) or animal origin (e.g., poultry meal). In the case of least-cost formulated diets, nutritionists have the option of using alternative protein ingredients that may or may not have an impact on pellet

quality (Briggs et al., 1999; Buchanan and Moritz, 2009). This is important because pellet quality is affected by pelleting conditions and heat-transfer reactions. Although protein denaturation is required for nutritional purposes, it is also affected by changes in temperature which may impact nutrient digestibility (Buchanan et al., 2010; Svihus and Zimonja, 2011). When the temperature increases, the tertiary structure of the protein unfolds and improves its digestibility (Moran, 1987; Scott et al., 1997; Dozier, 2001); nonetheless, when pressure is applied, the tertiary and quaternary structure of protein are denatured and inactivated (Svihus and Zimonja, 2011). Moreover, some proteins start to denature at 60 °C in the presence of excess moisture while others can tolerate higher temperature (Adams, 1991; Ludikhuyse et al., 2003). A common effect of high strenuous feed processing conditions may result in a Maillard reaction. The Maillard reaction takes place between amino acids and reducing sugars at high temperature and low moisture resulting in browning of the product and decreased nutritional content of the diets (Camire et al., 1990; Voragen et al., 1995; Thomas et al., 1998). In the case of individual amino acids, previous research has reported that high temperatures may result in loss of of cysteine, lysine, arginine, threonine, and serine (Papadopoulos, 1989), however, other studies have reported that pelleting temperatures of 72° C and 88° C have no effect on lysine survivability (Dale, 1992; Shipe et al., 2011). Regardless of the type or source of protein, it seems that the temperature, pressure, moisture, and friction exerted by the conditioner and the pellet mill die may have a significant impact on protein denaturation or digestibility as a consequence of feed processing.

Enzymes. An enzyme is a protein that accelerates chemical reactions but if the unfolding alters the shape of the active site, it causes enzyme inactivation. In the case of enzymes, high temperatures affect the binding with its substrate (Amerah et al., 2011; Chassaing et al., 2015).

The influence of high temperature on the efficacy of enzymes have been extensively studied and reviewed in broiler diets (Inborr and Bedford, 1994; Spring et al., 1996; Silversides and Bedford, 1999; Jensen, 2000; Amerah et al., 2011; Abdollahi et al., 2013; Pope and Fahrenholz, 2020). Spring et al. (1996) reported that cellulase, pentosanase, and fungal amylase lost > 90% of their activity when pelleting temperature increased from 60° C to 90° C. In agreement, Cowieson et al. (2005) reported that pelleting temperatures of 70° C and 85° C recover > 80% of xylanase and amylase. Previous research has reported that 90° C could be the highest temperature in which enzymes survive the pelleting process (Samarasinghe et al., 2000; Bedford et al., 2003; Cowieson et al., 2005). However, other factors such as conditioner retention time may also have a detrimental impact on enzyme recovery (Inborr and Bedford, 1994; Silversides and Bedford, 1999). Although current available coated enzymes are more heat-stable (Amerah et al., 2011), enzymes are highly thermo-sensitive ingredients susceptible to the current high temperatures used by the industry (Lahaye et al., 2004; Bedford and Cowieson, 2009; Amerah et al., 2011) thus, it is critical to understand the main locations of degradation within the conditioning and pelleting processes. The first step would be to consider that conditioner temperature is different from the temperature rise of pellets after exiting the pellet mill die. Pope and Fahrenholz (2020) evaluated the effect of conditioning temperature on phytase recovery and concluded that the primary point of denaturation for the particular phytase tested was the pellet mill die. The inclusion of enzymes is a common practice in broiler diets and are essential for nutrient digestibility. However, whether this denaturation occurs in the conditioner or the die requires further investigation. The determination of pellet temperature in-real time could be an alternative to monitor thermo-sensitive additives incorporated in broiler diets and support theories of heat rise at die exit due to friction and lower moisture.

Binders. Pellet binders have been suggested to improve pellet quality and binding of particles within pellets. Previous research has reported that pre-gelatinized starch can act as a binder and improve pellet quality (Wood, 1987; Zimonja and Svihus 2009). Despite the positive binding properties of starch, starch gelatinization may not be directly related to pellet durability or be greatly affected by temperature (Gilpin et al., 2002; Svihus and Zimonja, 2011). Angulo et al., (1996) reported the addition of sepiolite (hydrated magnesium silicate clay) as a pellet binder increased temperature of pellet at die exit in swine diets. In experiment 1 and 2, the inclusion of sepiolite product in the diet increased temperature of pellets by approximately 14 °C and 7° C, respectively (Angulo et al., 1996). In experiment 2, the finisher diet had a higher addition of fat thus the impact of the binder on pellet temperature was negligible due to lubrication (Angulo et al., 1996).

Fiber. Dietary fiber has the ability to interact with other nutrients in broiler diets and can be classified as soluble or insoluble fiber. Tejeda and Kim (2021) stated that variations in functionality and different roles in gut microbiota of broilers, makes it a complex component of broiler diets. In the literature reviewed by Svihus and Zimonja (2011), the authors concluded that heat treatments have a more pronounced effect on viscosity properties of soluble fibers than the actual amount recovered.

Vitamins. Vitamin stability is interesting because they have variations in their structure which makes some of them more susceptible to degradation than others. Previous research has reported that high temperatures can reduce retention of vitamins up to 50% and they can react with oxygen and light to reduce their activity (Beetner et al., 1974; Guzman-Tello and Cheftel, 1990; Britton, 1992; Anderson and Sunderland, 2002; Lešková et al., 2006). In contrast Lewis et al. (2015) reported that the concentrations of riboflavin, niacin, and vitamin D3 were not affected

by 2 conditioning temperatures (77° C vs. 88° C) and 3 conditioner retention times (15s, 30s, and 60s) in swine diets. Marchetti et al. (2009) reported that pyridoxine, folic acid, and vitamin B are more heat tolerant than vitamins K and C during pelleting. Therefore, vitamins react differently to a variety of cooking and extruding methods.

TEMPERATURE MONITORTING DURING THE PELLETING PROCESS

High temperatures may limit the survivability of enzymes and significant destruction of available nutrients or other feed additives. While mash feed can reach a temperature of approximately 75° C in the mixer, previous research has reported that the die friction can cause a temperature rise of approximately 15° C on pellets after being extruded by the die (Svihus et al., 2004; Svihus and Zimonja, 2011). However, the equipment design of a pellet mill system makes data collection laborious, hence alternative methodologies have been developed to measure hot pellet temperature (**HPT**). Temperature monitoring of a pelleting process can be contact or non-contact methods. Contact methods (e.g., temperature probes and RTDs) for measuring HPT in real-time are difficult and expensive to develop while non-contact methods have more simple designs and applications. Salas-Bringas (2007) used IR thermography to determine temperature rise during pelleting. The authors concluded that there is an increase in HPT once the pellets exit the die but non-contact methods may not provide an adequate estimate of die temperature and have difficulties operating in the harsh conditions within the pelleting chamber. Hence, a contact method could better predict the increase in HPT of pellets after accounting for die frictional forces.

Development of an In-Line Methodology to Measure Hot Pellet Temperature at the North Carolina State University (NCSU) Pellet Mill

Based on the literature reviewed, pellets may reach higher temperatures than the temperature readings obtained with insulated containers and/or thermometers/temperature probes. Being able to measure the temperature of pellets the moment they are extruded by the die is certainly a feasible idea but is difficult to implement practically. The main challenges to collect experimental measurements directly from the pellet mill die include die rotational speed (can range from 100 to 400 rpm) (Fairfield et al., 2005), pellet mill door enclosure, and steam generation inside the pelleting chamber. Therefore, this methodology would require the use of wireless temperature sensors that can record data over time or in relation to location, and equipment welding which could significantly increase the total cost of the project. Even though IR thermometers/guns provide wireless measurements, the steam coming out of the conditioner and the pointing or location of the gun may interfere with the accuracy of the temperature readings. In the case of wireless temperature sensors, they would need to be mounted on the surface of the die since it would provide most precise locations for the temperature readings of pellets as they are exiting the die. However, wireless sensors require a diagnostic unit connected via wireless communication lines to collect hot pellet temperature and send these readings to an external computer. Therefore, wireless sensors that could be mounted on the die surface and transmit temperature readings through the stainless-steel pellet mill door were non-existent which made the process more complicated. The next alternative for the methodology was to install a profile temperature probe with multiple sensing points connected to a data logger that could record the hot pellet temperature while running the pellet mill in normal manufacturing conditions. Ideally, the profile probe should be installed to where the sensing points of the probe

are nearly contacting the pellets as they are coming out of the die. Therefore, the only option was to mount the profile probe to a pellet mill knife as it provided a solid support against the die aggressiveness and allow the sensing points of the probe to be adjusted closer to the feed pellets. If the current available temperature probes are not ideal to measure hot pellet temperature, alternative sensors or technologies should be further explored.

KNOWLEDGE GAPS IN THE LITERATURE

Based on previous research, chicks may need a more uniformly mixed diet during the starter period. However, the majority of feed manufacturers and nutritionists are using the same mixing time to maintain mix uniformity throughout the starter, grower, and finisher periods of broilers. This could prove to be a large issue when manufacturing high tonnage of feed on a weekly basis. If a lower mix uniformity of feed does not decrease growth performance of birds in the grower and finisher periods, then a significant amount of mixing time can be saved in these high-feed manufacturing facilities, hence improving feed throughput and reducing labor hours. Mixing time could be adjusted according to age and feeding phase in order to improve feed manufacturing efficiency without a negative impact in overall live performance of broilers.

Previous research has often focused on the effects of NIR technologies on predicting nutrient variation and of ingredients. However, it is necessary to evaluate the effects of modern NIR sensors and equipment on feed quality parameters of broiler diets. Research evaluating the benchtop NIR or in-line applications as tools for predicting protein and moisture content has been conducted primarily in cereal grains while investigations of NIR applications on mixed feed is sparse.

In addition to mixing and NIR applications, most of the pelleting research in broiler diets evaluate the effects of conditioning temperatures on feed thermostability and enzyme

recovery. However, novel methodologies or technologies to assess hot pellet temperature fluctuations and the impact of the pellet mill die on feed quality are often neglected. Published literature has not determined an in-line method or device to measure hot pellet temperature in broiler diets, which has been reported to influence thermos-sensitive ingredients. In order to address these knowledge gaps in the literature, 3 experiments were conducted to evaluate the effects of mixing and pelleting technological applications on feed quality parameters and broiler growth performance. The first experiment evaluated the effects of mix time on coefficient of variation (mix uniformity), individual body weight uniformity, and broiler growth performance during the starter, grower, and finisher periods. The second experiment evaluated the interactive effects of marker selection, mix time, batch size, and In-line near infrared spectroscopy on the coefficient of variation (mix uniformity) of broiler diets. The third experiment evaluated the comparison between an in-line implementation of a temperature profile probe-based tool vs. three non-contact methodologies to monitor hot pellet temperature in broiler diets.

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CHAPTER 1

Effects of mix time on coefficient of variation (mix uniformity), body weight uniformity, and broiler growth performance during the starter, grower, and finisher periods

SUMMARY

Most feed manufacturers in the United States use the same mixing time (and thus mix uniformity) throughout the growing period regardless of age and consumption patterns. However, research evaluating the optimum mixing time requirements on growth performance of broilers during the starter, grower, and finisher periods of broilers is sparse. Therefore, the objective of this study was to evaluate the effects of mix uniformity on broiler growth performance and body weight uniformity (expressed as CV) from 1 to 42 d of age. Feed was manufactured utilizing a 1815-kg counterpoise ribbon mixer. Two batches of feed were mixed for 4.5 min (3 min dry mix and 90 s of wet mix) and 30 s (0 s dry mix and 30 s wet mix) to obtain a Uniform (UM) and a Non-uniform (NUM) mix, respectively. The experiment consisted of 4 dietary treatments in which broilers received different mix uniformities: 1) UM from 1-42 d, 2) UM from 1-28 d and NUM from 28-42 d, 3) UM from 1-14 d and NUM from 14-42 d, and 4) NUM from 1-42 d. Mix uniformity did not influence BW, feed intake (**FI**), FCR, and individual bird BW CV from 1 to 42 d of age. These data indicated that diets with a reduced mix time and uniformity may not influence growth performance and BW uniformity during the grower and finisher periods.

DESCRIPTION OF PROBLEM

Mix uniformity of broiler diets is a quality parameter that can be controlled depending on the time that ingredients reside in the mixer. Previous research has reported that mixing uniformity is indirectly proportional to mixer capacity and mixing time (Wicker and Poole 1991; McCoy et al., 1994). Typically, an adequate mix time or uniformity is obtained when a defined marker in the diet has a coefficient of variation (**CV**) < 10% (Beumer, 1991). Poultry consume lower amounts of feed compared to swine and cattle, hence a proper mixing time and nutrient homogeneity have been thought to be required to maximize nutrient consumption and optimize their growth performance (Creger, 1957; McCoy et al., 1994). Currently, the total mix time used in broiler diets throughout the growing period is often 3 to 5 min to maintain nutrient homogeneity and compliance with feed manufacturing regulations. A good quality mixed and uniform diet has been reported to be essential for growth in nursery pigs and young chicks during the starter period (Ensminger et al., 1990; Traylor et al., 1994; Clark et al., 2007). Nevertheless, if mixing time could be reduced, particularly during the grower and finisher periods, it could be possible to improve feed throughput and reduce labor in high-volume feed manufacturing facilities. Moreover, mix times ≤ 4 min have been reported to have no detrimental impact in growth performance of swine and poultry (Traylor et al., 1994; Ciftci and Ercan 2003; Paulk et al., 2015). Although previous research has evaluated the effects of mix time on broiler growth performance, differences in mixing equipment (Ciftci and Ercan 2003), post mixing by pelleting and cooling processes (Reese et al., 2017), batch size (Wilcox and Unruh, 1986; Wicker and Poole, 1991), and marker selected for CV determination (Clark et al., 2007) have produced inconsistent results. The evaluation of mix uniformity in specific grow out periods of broilers is crucial to determine adequate mixing time without compromising growth performance.

Therefore, a study was conducted to evaluate the effects of mix time on CV (mix uniformity), body weight uniformity, and broiler growth performance from 1 to 42 d of age.

MATERIALS AND METHODS

All procedures involving live birds were approved by North Carolina State University Institutional Animal Care and Use Committee (PRN 21-393-A).

Husbandry Practices

In this experiment, Ross × Ross 308 male chicks (Aviagen North America, Huntsville, AL) were obtained from a resident broiler breeder flock housed at NCSU at 1 d of age. A total of 640 male broiler chicks were feather-sexed, weighed and randomly distributed among 40 floor pens (16 birds/pen; 0.14 m^2/bird) in an environmentally controlled room. Individual birds and feed were weighed to determine body weight (**BW**), feed intake, and feed conversion ratio (**FCR**) at 1, 14, 28, and 42 d of age. The facility was equipped with exhaust fans, forced-air heaters, cooling pads, and electronic controllers to manage temperature and ventilation. Each pen was 122 cm in width, 187 cm in length, and 91 cm in height and was equipped with nipple drinkers and 1 tube feeder. Feed and water were offered *ad libitum* throughout the experimental periods. The lighting program consisted of 23L:1D from 1 to 7 d, 21L:3D from 8 to 20 d and 16L:8D from 21 to 42 d. The room temperature was 35°C at placement, 31.3°C from 2 to 5 d, 29.4°C from 6 to 14 d, and 28.3°C from 15 to 23 d, 26.7ºC from 24 to 28 d and 23.9ºC from 29 to 42 d.

Feed Formulation, Manufacture, and Experimental Design

Broiler diets were formulated to meet or exceed the NRC suggested minimum nutrient requirements of broilers (NRC, 1994) (Table 1). Dietary treatments were formulated with corn and soybean meal (SBM) as the primary ingredients. Six batches of feed were mixed for 4.5 min

(3 min dry mix and 1.5 min of wet mix) and 0.5 min (0 min dry mix and 0.5 min wet mix) to obtain a Uniform (**UM**) and a Non-uniform (**NUM**) mix, respectively. Each pen was randomly assigned to 1 of 4 dietary treatments: 1) UM from 1 to 42 d of age, 2) UM from 1 to 28 d of age and NUM from 28 to 42 d of age, 3) UM from 1 to 14 d of age and NUM from 14 to 42 d of age, and 4) NUM from 1 to 42 d of age represented by 10 replicate pens. To create the experimental treatments, dry ingredients were blended in a double shaft counterpoise ribbon mixer with a 1815-kg full load capacity, inlet for major and minor ingredients, a top access door for additional ingredients (hand-adds), and discharge gates (Model TRDB126060, Hayes and Stolz, Forth Worth, TX). During the starter and grower periods, UM and NUM diets were batched as to utilize half capacity of the mixer on a weight basis (454 kg/batch). The UM and NUM finisher diets were batched as 454 and 908 kg/batch, respectively. All fat in the experimental diets was in the form of poultry oil (1%). In the UM diets, dry mix time began after all major, minor, and hand-add dry ingredients were added at the instant the mixer began to run. After the completion of the dry mix cycle, the wet mix cycle began and continued while poultry oil was sprayed into the mixer. In the NUM diet, the mixer was manually controlled, and the mix time was determined based on the amount of time required to add poultry oil into the batch of feed (0.5 min). The major and minor ingredients (corn, SBM, and poultry by product meal) were discharged into the mixer from the major batch scale. All micro ingredients were individually preweighed into a barrel and added to the mixer at the top access door to prevent a complete dry mix cycle (0 min). For both mix uniformities, the mixing time started when the last ingredient was added to the mixer and ended with mixer discharge, hence it does not include discharge time. Discharge time of the major and micro scales was approximately 0.6 and 0.5 min,

respectively. The experimental diets were offered in mash form to prevent additional mixing through pelleting and conveying feed systems.

After mixing, the mixer was stopped, the discharge gate was opened, and the mixed feed was conveyed by a drag conveyor (4.3 m), elevated (3.7 m), and dropped into a folding bulk container (908-kg full load capacity). Ten feed samples (3 kg of each) were collected from the discharge end using an open-top container (5 gal) and then divided into smaller aliquots for laboratory analysis. Mash samples were collected at equally spaced time intervals (approximately 0.25 min and 0.13 min/sample) according to previously determined mixer discharge time. Mixed feed discharge time was 2.5 min/908 kg and 1.25 min/454 kg batch size of feed. The NUM diet was packaged continuously, and samples were not split to prevent potential further mixing upon discharge, storage, and laboratory analysis. To analyze mix uniformity, representative subsamples of the UM and NUM diets were analyzed, and mixer CV was determined with the use of a "marker" or nutrient (Table 2). The inclusion rate of all markers in the dietary treatments was less than or equal to 0.5%.

Whole corn was ground in a hammermill (Model 1522, Roskamp Champion, Waterloo, IA) equipped with 2.4-mm and 3.2 mm-screens to achieve an average particle size of 332 µm. All nutrients and markers selected to determine mixer CV were analyzed for particle size analysis: sodium chloride (391 µm), L-Lysine-HCl (78%) (523 µm), D-L Methionine (99%) (194 µm), Phytase (811 µm), Microtracers Red #40 (203 µm), and Microtracers Blue #40 (209 µm) (Microtracers Inc., San Francisco, CA) (Figure 1.1). Particle size was determined using a 15-sieve stack with rubber balls, bristle sieve cleaners, and with US sieve numbers 4, 6, 8, 12, 16, 20, 30, 40, 50, 70, 100, 140, 200, 270, and pan. A Ro-Tap shaker (Model RX-29 W.S. Tyler's Ro-Tap®, Mentor, OH) was used to sift 100 ± 5 g samples for 10 min. Before sifting the

sample, 0.5 g of flow agent (Model SSA-58 Gilson's Inc. Sieving Aid, Lewis Center, OH) was added. Geometric mean particle size by mass (D_{gw}) and the geometric standard deviation of particle diameter by mass (S_{gw}) were determined using the quantity of material retained on each sieve following the ASABE method S319.4 (ASABE, 2009).

Measurements

Subsamples of mash feed were analyzed for sodium chloride, L-Lysine-HCl (78%), D-L Methionine (99%), Phytase, Microtracers RedF #40, and Microtracers BlueF #40, and a coefficient of variation (CV) was calculated for each of the mixing uniformities fed to broilers (Table 2). The industry standard for CV to obtain a uniform mix is less than 10%. The coefficient of variation is calculated using the following formula:

Mean (μ) = $\sum x_i / n$

Standard Deviation (s)= $\sqrt{\sum (x_i - \mu)^2 / (n-1)}$

Percent Coefficient of Variation (% CV) = $(s / \mu) * 100$

Feed intake and BW by pen were recorded at 1, 14, 28, and 42 d of age. Birds were observed twice daily, mortalities were removed, and their BW was included in the FCR calculation. The incidence of mortality was recorded daily. Individual BW uniformity by pen was expressed as the CV of BW at 14, 28, and 42 d of age.

Statistical Analyses

A randomized complete block design was employed with pen location as the blocking factor. Each treatment was represented by 10 replicate pens with pen being the experimental unit. Mortality data were subjected to arcsine transformation before analysis. Data were analyzed as a one-way ANOVA using the GLM procedure of JMP software (JMP, 2010) with the following model:

$$Yi\ j = \mu + \mathrm{T}i + \varepsilon ij$$

Where $Yi\ j$ = observed response of the broilers in the pen; μ = is the overall mean; Ti = fixed effect of mixing uniformity treatment; and εij = residual error when the pen was regarded as an experimental unit, $\varepsilon ij\ N(0, \sigma^2_{\varepsilon})$. The mean values among 4 mixing uniformity treatments were compared using the Tukey's honestly significant different procedure with the significant level at $P \leq 0.05$ unless otherwise indicated. Linear and quadratic effects were not considered during data analysis as dietary treatments were not equally spaced.

RESULTS AND DISCUSSION

The mixer CV of tracer and selected markers, as determined by mix uniformity (total mix time), are reported in Table 2. Although the present study was designed to evaluate the effects of mix uniformity on broiler growth performance, previous research has reported the effects of sampling location, marker selection, analytical techniques, and the physical properties of markers on the mixer CV of broiler diets (Creger, 1957; Pfost et al., 1966; Wilcox and Unruh, 1986; McCoy et al., 1994; Clark et al., 2007). The selected markers and tracers should report a mixer CV >10% in the NUM diet (0.5 min) compared with a longer operating mix time but conflicting results were observed. Sodium chloride (salt) can be analyzed through chemical or test strip method, the latter being lower-cost and providing faster results (Ciftci and Ercan 2003). Salt concentration may be determined by measuring either the sodium or chloride ion. However, the inclusion of L-Lysine HCl and choline chloride in diets may interfere with the interpretation of the results when chloride is measured (Clark et al., 2007), while the inclusion of sodium bicarbonate may interfere when samples are analyzed for sodium. In this experiment, test strips were used to measure chloride concentration, and both L-Lysine HCl and choline chloride were included in the experimental treatments to replicate broiler diets fed commercially. This could

have had an impact on the uniformity test and hence the readings and conversion to %NaCl may not be strictly accurate. In addition, inconsistencies were observed in the results of mixer CV's based on supplemented D-L Methionine (99%), L-Lysine HCl (78%), and phytase at different mix times and experimental periods. The use of all Fe markers (Microtracers®) resulted in a CV >10% when the total mix time was 0.5 min except the Microtracer Blue #40 in the grower diet. Rocha et al. (2015) reported that the use of Manganese Sulfate (MnS), Copper Chloride (CuCl), Zinc Sulfate (ZnS), and sodium chloride (NaCl) can produce confounding CVs as a result of other minor and micro ingredients containing Mn, Cu, Zn, and Cl. Therefore, D-L Methionine, L-Threonine, L-Lysine HCl (99%), and Microtracer Red #40 are suitable markers to evaluate mix uniformity in dry feed. The suitability of the CV analysis methods used in broiler diets is influenced by analytical assays, the inclusion rate of feed ingredients, common nutrient sources, and markers or tracer selection, which can result in over-or underestimation of mix uniformity. McCoy et al. (1994) concluded that broilers could be fed diets with a mixer CV of 20% without a negative impact on growth performance. Therefore, the effects of CV methodologies in selected markers or tracers in relation to mixing time require further evaluation to determine a suitable mix uniformity in broiler diets. However, in the present study, the authors acknowledge that although not all the mixer CVs results obtained were considered "poor" (> 10%), the mix time could not be decreased any further with the batching and mixing equipment available. In addition, the type of mixer and/or mixer capacity used in previous studies (which can be found in current feed facilities) are not necessarily representative of the modern mixers installed in new and/or renovated facilities. Furthermore, even with the mixing equipment used and with higher CV values, there was not a detrimental impact on the animal's growth performance which will be discussed in the following section.

Mix uniformity of mash diets did not influence BW, BWG, FI, FCR, or the incidence of mortality from 1 to 14 d of age ($P > 0.05$) (Table 3 and 4). McCoy et al. (1994) reported similar effects on broilers fed diets containing different mixer revolutions (mix times) without a negative effect on growth performance during the starter period. In contrast, Groesbeck et al. (2007) reported that average daily gain (ADG) increased from 190 to 280 g and Gain:Feed from 0.71 to 0.90 as mix time increased from 0 min to 5.5 min at 14 d of age in pigs. Similarly, Traylor et al. (1994) reported an increase in ADG and average daily feed intake (ADFI) on nursery pigs by increasing mix time from 0 to 0.5 min. Even though a minimal increase in mix time improved growth performance, the authors stated that manufacturing diets with a significantly shorter mix time than the standard is not suitable for optimum growth in young pigs. Previous research has reported that mix uniformity has a greater impact on younger chicks and pigs because they consume less feed during the starter period when compared to periods thereafter (Ensminger et al., 1990; Traylor et al., 1994). However, in the present study, feeding a poorly mixed diet during the starter period did not have a negative impact on growth performance of young chicks.

Mix uniformity of mash diets did not influence BW, BWG, FI, FCR, or the incidence of mortality from 28 to 42 d of age ($P > 0.05$) (Table 3 and 4). Similarly, Traylor et al. (1994) reported that mix times of 0, 0.5, 2, and 4 min had no effect on growth performance of finishing pigs. The authors stated that the experimental treatments in their study had additional mixing time through the feed conveying systems which could have increased uniformity of the 0 min mix time. Ciftci and Ercan (2003) reported no differences in BWG, FI, FCR, and mortality on broilers fed diets containing mix times of 0.20, 0.59, and 3.75 min at 42 d of age. These data indicated that, although a detrimental impact in growth performance was expected by decreasing mix time from 4.5 to 0.5 min, broilers tolerated a less uniform mix in the late stages of their

feeding phases. According to the Food and Drug Administration's (FDA) Good Manufacturing Practices, it is expected that animal feed in compliance with regulations will have a suitable uniformity of nutrients in diets and feed additives (Traylor et al., 1994; Muirhead 2006), but rules do not specifically identify required mixer CV values. Therefore, the total mix time for broiler diets fed during the grower and finisher periods may be reduced without compromising homogeneity of nutrients in finished feed.

Mix uniformity of mash diets did not influence CV of 14, 28, and 42 d of age BW ($P > 0.05$) (Table 5). A recent study reported no differences in CV of 27 d and 33 d BW in finishing pigs fed diets containing mix times of 0, 0.5, 2, and 6 min (Paulk et al., 2015). In addition, the authors reported that increasing mix time from 0 to 6 min reduced the CV of markers added in feed such as salt and chromium by 39% and 36%, respectively. However, these improvements in ingredient variation were not sufficient to impact BW uniformity of finishing pigs. Ciftci and Ercan (2003) reported similar effects on broilers fed diets containing different mix times (0.20, 0.59, and 3.75 min) without negatively affecting the CV of 42 d BW. Previous research has reported that nutrient deficiency, variation in genotype and environmental conditions inside a broiler house have a significant impact on BW uniformity within a flock of broilers (Al Homidan et al., 1998; Gous, 2018). Gous (2018) concluded that broilers fed diets containing higher inclusion rates of protein and vitamins and cooler house environment results in increased BW uniformity of broiler flocks. In addition, Xu et al. (2015) reported that feeding mash diets decreases BW uniformity compared with crumbled and pelleted diets. In the present study, diets were fed in mash form to reduce additional mix time by feed conveying systems and a selected feed allocation of the packaged NUM diets was used to increase non-uniform nutrient intake and uniformity within the flock. However, the renovated conditions of the broiler house and cool

weather conditions provided a steady microenvironment and stimulated feed intake of broilers which may have precluded differences in BW uniformity.

Even though birds fed the UM diets were expected to outperform those fed NUM diets during the starter period, the impact of the NUM diets on growth performance was negligible in subsequent phases. The difference in results among studies could be attributed to the type of mixer used, mixer capacity, and marker selection to determine mixer CV. Previous research has reported that the wear of mixer ribbons, bulk density of ingredients, marker particle size, sample preparation, and mixer fill could be sources of variation in diets homogeneity that can lead to modifications in total mix time (Herrman and Behnke, 1994; McCoy et al., 1994; Groesbeck et al., 2007; Clark et al., 2007). These data demonstrated that diets with a total mix time of 0.5 min can be fed to broilers during the grower and finisher periods without compromising growth from 28 to 42 d of age.

CONCLUSIONS AND APPLICATIONS

1. The mixer CVs values obtained from the majority of the selected markers did not exceed the 10% mixer CV standard even in poorly mixed diets. Therefore, efforts to obtain particularly high mixer CVs may not be possible especially in facilities with modern, high-speed mixers and may not have a negative impact on the animal's growth performance.
2. Broilers were able to consume a NUM diet without any adverse effects on BW, BWG, FI, FCR, mortality, and body weight uniformity from 1 to 42 d of age.
3. In the present study, mix uniformity (mix time) of mash diets did not compromise growth performance and BW uniformity during the grower and finisher periods, which indicates that a shorter mix time is suitable for broilers in subsequent phases. Therefore, future

research should evaluate the effects of feeding pelleted diets with reduced mixer CV on growth performance during the grower and finisher periods of broilers.

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Quantum Blue to which the Quantum Blue protein in the diluted supernatant binds. After washing steps, enzyme (horseradish peroxidase)-labelled Quantum Blue antibody was added to the wells. After another series of washing steps the substrate for the horseradish peroxidase was added and the subsequent color reaction was measured at 415/630 nm. The amount of color produced is proportional to the amount / activity of Quantum Blue in the original feed sample. Results are calculated from a calibration curve prepared from different dilutions of a Quantum Blue preparation, with a known activity as determined by the Quantum Blue product analysis. Units of activity are traced to the Quantum Blue product method and activity is expressed as FTU/kg.

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isolate the Microtracer(s) F, 3) Transfer the magnetic material to a 30 ml analytical scoop using a fantail brush, 4) “Demagnetize” the material with a bulk tape eraser and then sprinkle the material to a 150-185 mm #1 Whatman filter paper, 5) Sprinkle/spread the demagnetized particles as uniformly as possible, 6)Wet the filter paper with 75% ethanol, 7) When spots begin to visually appear in the filter paper, place the filter paper into a preheated oven (149°C) or hot plate, 9) Leave the filter paper until dry, 10) Count the colored spots by circling the spots (Handcount) or download the Microtracers® App to use the spot counter function. 11) Determine the CV for each set of samples using Poisson statistics and chi-squared calculations.

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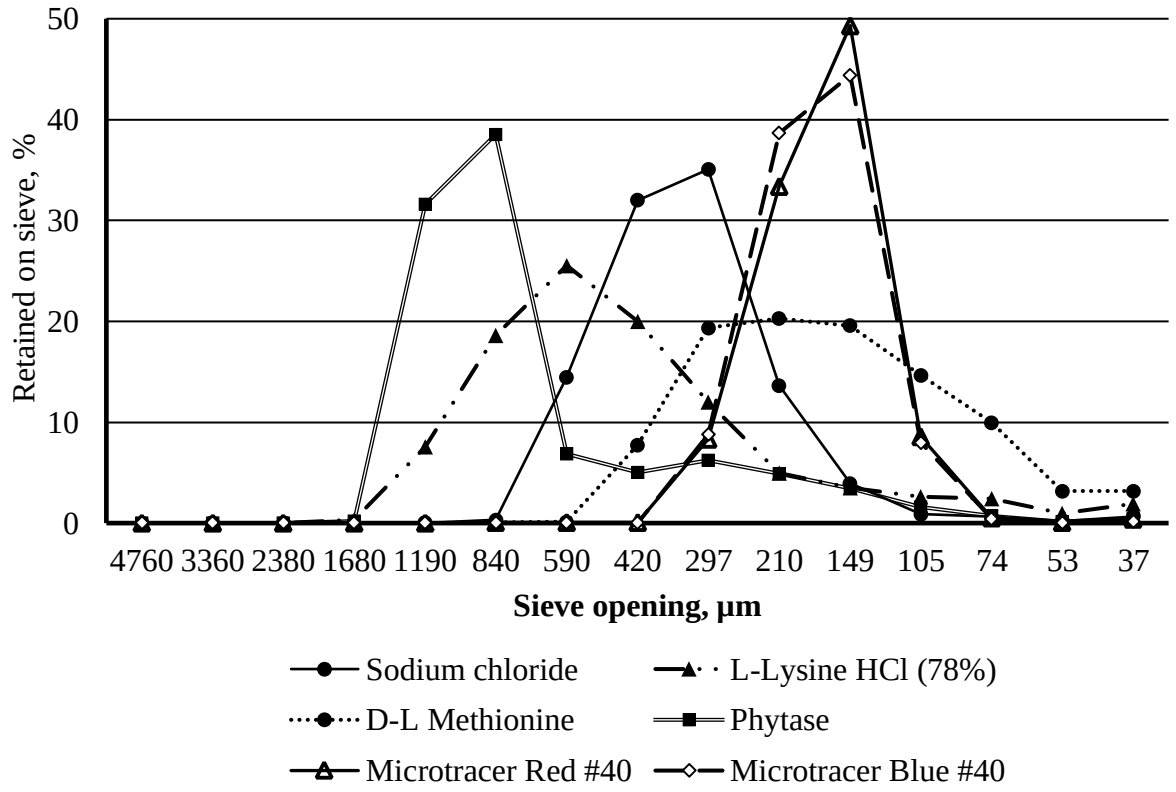

Figure 1.1 The geometric mean diameter by mass (Dgw) and particle size distribution of markers and nutrients before mixing was determined. The inclusion rate of all markers in the dietary treatments was less than or equal to 0.5%. Ross × Ross 308 male broilers were fed with diets containing a Uniform (UM) and Non-Uniform (NUM) mix during the starter, grower, and finisher periods. Dietary treatments consisted of 1) Uniform Mix (1 to 42 d) (n =10), 2) Uniform Mix (1 to 28 d) and Non-Uniform Mix (28-42 d)(n =10), 3) Uniform Mix (1 to 14 d) and Non-Uniform Mix (14-42 d)(n =10), and 4) Non-Uniform Mix (1-42 d)(n=10). The UM diets were mixed for 4.5 min (3 min dry mix and 1.5 min of wet mix) and the NUM diets were mixed for 0.5 min (0 min dry mix and 0.5 min wet mix) in a counterpoise ribbon mixer with a 1815-kg full load capacity. All nutrients and markers selected to determine mixer CV were analyzed for particle size analysis: sodium chloride (391 µm), L-Lysine-HCl (78%) (523 µm), D-L Methionine (99%) (194 µm), Phytase (811 µm), Microtracers Red #40 (203 µm), and Microtracers Blue #40 (209 µm).

Table 1.1 Ingredient and nutrient composition of dietary treatments fed to Ross × Ross 308 male broilers from 1 to 42 d of age.

Ingredient, % "as-fed"	Starter	Grower	Finisher
Corn	58.89	66.28	71.41
Soybean Meal, 48 % Crude Protein	31.75	24.87	20.11
Poultry by Product Meal	5.00	5.00	5.00
Poultry Oil	1.00	0.90	0.98
Monocalcium phosphate, 21% P	0.90	0.65	0.39
Calcium carbonate	0.84	0.75	0.66
Sodium chloride	0.50	0.47	0.45
D-L Methionine (99%)[1]	0.30	0.25	0.20
Trace mineral premix[2]	0.20	0.20	0.20
Choline Chloride	0.20	0.20	0.20
L-Lysine-HCl (78%)	0.15	0.17	0.16
L-Threonine	0.10	0.09	0.07
Vitamin Premix[3]	0.05	0.05	0.05
Selenium, 0.06%[4]	0.05	0.05	0.05
Salinomycin sodium[5]	0.05	0.05	0.05
Phytase[6]	0.01	0.01	0.01
Microtracers (Red[7] and Blue[8] #40)	0.01	0.01	0.01
	100.00	100.00	100.00
Calculated analysis, % (unless otherwise noted)			
AME_n, kcal/kg	3,000	3,070	3,130
Crude Protein	22.83	20.17	18.28
Digestible Lys	1.37	1.21	1.08
Digestible SAA[9]	1.01	0.90	0.80
Calcium	1.00	0.90	0.80
Available phosphorus	0.50	0.45	0.40

Abbreviation: AME_n, apparent metabolizable energy

[1]Donated by Evonik Corporation

[2]Mineral premix include per kg of diet: Mn (manganese sulfate), 120 mg; Zn (zinc sulfate), 120 mg; Fe (iron sulfate monohydrate), 80 mg; Cu (tri-basic copper chloride), 10 mg; I (ethylenediamine dihydriodide), 2.5 mg; and Co (cobalt), 1 mg.

[3]Donated by DSM Nutritional Products North America. Vitamin premix includes per kg of diet: Vitamin A (Vitamin A acetate), 6600 IU; Vitamin D (cholecalciferol), 1980 IU; Vitamin E (DL-alpha tocopherol acetate), 33 IU; menadione (menadione sodium bisulfate complex), 2 mg; Vitamin B12 (cyanocobalamin), 0.02 mg; folacin (folic acid), 1.1 mg: D-pantothenic acid (calcium pantothenate), 11 mg; riboflavin (riboflavin), 6.6 mg; niacin (niacinamide), 55 mg; thiamin (thiamin mononitrate), 2 mg; D-biotin (biotin), 0.13 mg; and pyridoxine (pyridoxine hydrochloride), 4 mg.

[4]Selenium premix provided Se at 0.3 mg/kg of feed.

[5]Bio-Cox® 60 provided salinomycin sodium at 60g/ton of feed.

[6]Quantum® Blue 5G (Donated by AB Vista Feed Ingredients, Marlborough, UK) provides per kg of diet: 500 FTU/kg of phytase activity.

[7]Microtracer Red #40 Fe marker added at 50g/ton.

[8]Microtracer Blue #40 Fe marker added at 50g/ton.

[9]SAA = Total sulfur amino acids.

Table 1.2 Mixer coefficient of variation (CV) of nutrients and markers selected of dietary treatments fed to Ross × Ross 308 male broilers from 1 to 42 d of age.

Marker, CV %[1]	Mix time (min)[2]						Method of Analysis
	0.5			4.5			
	Days of age[3]						
	14	28	42	14	28	42	
DL-Methionine (99%)	4.94	5.18	8.32	5.62	6.15	9.43	AOAC 999.12/Free AA extraction
L-Lysine HCl (78%)	5.19	8.99	8.31	7.55	8.67	5.01	AOAC 999.13
Chloride ion (as sodium chloride)	10.70	3.85	5.92	3.85	6.29	3.80	Quantab® Chloride Titrator
Phytase[4]	19.95	16.18	21.04	28.16	23.50	37.05	ELISA method
Microtracer Red #40 (count)[5]	12.19	12.48	14.55	7.81	3.43	6.39	Microtracer Rotary Detector (Handcount)
Microtracer Blue #40 (count)[6]	12.85	7.13	15.68	4.42	4.18	8.03	

[1]CV= Coefficient of variation (CV = (Standard Deviation/Mean) *100). Mash samples (3 kg of each) were collected at equally spaced intervals using an open-top container (5 gal) (n=10) and then divided into smaller aliquots for laboratory analysis. Mixed feed discharge time was 5 min/908 kg and 2.5 min/454 kg batch size of feed.

[2]Non-Uniform Mix (NUM): Batches of feed were mixed for 0.5 min (0 min dry mix and 0.5 min wet mix). All of the ingredients were added as hand-adds. Mixed feed was packaged continuously in a collapsible black container (908 kg full capacity) and samples were not split to prevent potential further mixing upon discharge, storage, and laboratory analysis.

Uniform Mix (UM): Batches of feed were mixed for 4.5 min (3 min dry mix and 1.5 min of wet mix).

[3]During the starter (14 d) and grower periods (28 d), UM and NUM diets were batched as to utilize half capacity of the mixer on a weight basis (454 kg/batch). The UM and NUM finisher (42 d) diets were batched as 454 and 908 kg/batch, respectively.

[4]Quantum® Blue 5G (AB Vista Feed Ingredients, Marlborough, UK) provides per kg of diet: 500 FTU/kg of phytase activity.

[5]Microtracer Red #40 Fe marker added at 50g/ton.

[6]Microtracer Blue #40 Fe marker added at 50g/ton.

Table 1.3 Growth performance of Ross × Ross 308 male broilers fed with diets containing varying mix uniformities from 1 to 42 d of age.

Item	BW, g/bird[4]			Feed intake, g/bird			FCR, g:g[5]			Mortality, %[7]		
	Days of age											
Dietary Treatments[1]	14	28	42	14	28	42	14	28	42	14	28	42
Uniform Mix (1-42 d)[2]	493	1,694	3,158	544	2,210	4,830	1.215	1.342	1.548	0.0	0.6	0.6
Uniform Mix (1-28 d)	491	1,681	3,134	536	2,178	4,805	1.205	1.340	1.550	1.2	1.3	0.6
Uniform Mix (1-14 d)	481	1,680	3,193	543	2,194	4,866	1.255	1.343	1.545	1.9	0.0	0.0
Non-Uniform Mix (1-42 d)[3]	483	1,667	3,169	538	2,199	4,851	1.236	1.358	1.544	0.0	0.6	1.3
SEM[6]	9	21	34	7	24	64	0.027	0.011	0.009	0.008	0.006	0.008
P-value	0.692	0.840	0.678	0.806	0.823	0.917	0.577	0.626	0.965	0.253	0.555	0.722

[a-b]Means within a column with different superscripts differ significantly ($P < 0.05$).
[1]Treatments consisted of diets with different mix uniformities: 1) Uniform Mix (1 to 42 d), 2) Uniform Mix (1 to 28 d) and Non-Uniform Mix (28-42 d), 3) Uniform Mix (1 to 14 d) and Non-Uniform Mix (14-42 d) and 4) Non-Uniform Mix (1-42 d).
[2]Uniform Mix: Batches of feed were mixed for 4.5 min (3 min dry mix and 1.5 min of wet mix).
[3]Non-Uniform Mix: Batches of feed were mixed for 0.5 min (0 min dry mix and 0.5 min wet mix). All of the ingredients were added as hand-adds.
[4]BW= Body weight.
[5]Feed conversion ratio was corrected for mortality.
[6]SEM= Standard error of the means for mix uniformity effect (n=10).
[7]Mortality values were arcsin transformed.

Table 1.4 Period growth performance of Ross × Ross 308 male broilers fed with diets containing varying mix uniformities from 1 to 42 d of age.

Item	BW gain, g/bird[4]			Feed intake, g/bird			FCR, g:g[5]		
	Days of age								
Dietary Treatments[1]	1-14	14-28	28-42	1-14	14-28	28-42	1-14	14-28	28-42
Uniform Mix (1-42 d)[2]	448	1,201	1,464	544	1,666	2,620	1.215	1.392	1.789
Uniform Mix (1-28 d)	446	1,190	1,454	536	1,642	2,627	1.205	1.391	1.792
Uniform Mix (1-14 d)	435	1,199	1,513	543	1,651	2,672	1.255	1.379	1.766
Non-Uniform Mix (1-42 d)[3]	438	1,184	1,502	538	1,661	2,651	1.236	1.406	1.744
SEM[6]	9	18	26	7	20	44	0.027	0.014	0.020
P-value	0.675	0.901	0.329	0.806	0.842	0.836	0.577	0.633	0.324

[a-b]Means within a column with different superscripts differ significantly ($P < 0.05$).
[1]Treatments consisted of diets with different mix uniformities: 1) Uniform Mix (1 to 42 d), 2) Uniform Mix (1 to 28 d) and Non-Uniform Mix (28-42 d), 3) Uniform Mix (1 to 14 d) and Non-Uniform Mix (14-42 d) and 4) Non-Uniform Mix (1-42 d).
[2]Uniform Mix: Batches of feed were mixed for 4.5 min (3 min dry mix and 1.5 min of wet mix).
[3]Non-Uniform Mix: Batches of feed were mixed for 0.5 min (0 min dry mix and 0.5 min wet mix). All of the ingredients were added as hand-adds.
[4]BW= Body weight.
[5]Feed conversion ratio was corrected for mortality.
[6]SEM= Standard error of the means for mix uniformity effect (n=10).
[7]Mortality values were arcsin transformed.

Table 1.5 Body Weight Uniformity (expressed as CV) of Ross × Ross 308 male broilers body weight fed with diets containing varying mix uniformities from 1 to 42 d of age.

Item	CV, %[4]		
	Days of age		
Dietary Treatments[1]	14	28	42
Uniform Mix (1-42 d)[2]	13	10	12
Uniform Mix (1-28 d)	16	12	14
Uniform Mix (1-14 d)	17	13	14
Non-Uniform Mix (1-42 d)[3]	16	11	12
SEM[6]	2	1	1
P-value	0.557	0.302	0.569

[a-b]Means within a column with different superscripts differ significantly ($P < 0.05$).
[1]Treatments consisted of diets with different mix uniformities: 1) Uniform Mix (1 to 42 d), 2) Uniform Mix (1 to 28 d) and Non-Uniform Mix (28-42 d), 3) Uniform Mix (1 to 14 d) and Non-Uniform Mix (14-42 d) and 4) Non-Uniform Mix (1-42 d).
[2]Uniform Mix: Batches of feed were mixed for 4.5 min (3 min dry mix and 1.5 min of wet mix).
[3]Non-Uniform Mix: Batches of feed were mixed for 0.5 min (0 min dry mix and 0.5 min wet mix). All of the ingredients were added as hand-adds.
[4]CV= Coefficient of variation (CV = (Standard Deviation/Average Pen Body Weight) *100)
[5]SEM= Standard error of the means for mix uniformity effect (n=10).

CHAPTER 2

The effects of marker selection, mix time, and In-line near infrared spectroscopy (NIR) on the coefficient of variation (Mix Uniformity) or broiler diet

SUMMARY

Previous research has reported that mixing uniformity is essential to maximize growth performance of animals with a low daily intake, such as poultry. However, differences in diet formulations, batch size, marker selection, and sample preparation could be sources of variation which may lead to over-or underestimation of mix uniformity. The objective of this study was to evaluate the effects of marker selection, mix time, batch size, and In-line near infrared (**NIR**) spectroscopy on mixer CV of broiler diets. Twelve batches of feed were mixed for 4.5 min (3 min dry mix and 90 s of wet mix) and 30 s (0 s dry mix and 30 s wet mix) to obtain a uniform and a non-uniform mix, respectively. The experiment constituted a 2×2×4 factorial arrangement of 2 mix times, (4.5 and 0.5 min), 2 batch sizes (908 and 1815 kg), and 4 methodologies to evaluate mixer performance (sodium chloride, Microtracers (Red#40 and Blue#40), and In-line NIR). For main effects, there were no statistical differences between batch sizes on CV ($P > 0.05$). However, a mix time of 4.5 min and the In-line NIR generated CVs less than 10% compared to 0.5 min, sodium chloride, and the Microtracers. Interactions were apparent ($P < 0.05$) only for methodology and total mix time. The sodium chloride method reported CV values greater than 10% regardless of mix time. These data indicated that mixer CV differed depending on total mix time and methodology used.

DESCRIPTION OF PROBLEM

Typically, an adequate mix uniformity has been considered to have been achieved when a defined nutrient or additive in the diet has a coefficient of variation (**CV**) ≤ 10%. However, differences in total mix time, marker selection, equipment design, sampling method, and mixer fill have been reported to have an impact on mix uniformity (Creger, 1957; Wicker and Poole, 1991; McCoy et al., 1994; Ciftci and Ercan 2003; Clark et al., 2007; Reese et al., 2017). Currently, the feed manufacturing and poultry industries perform laboratory assays on medications, amino acids, minerals, and chloride ion to evaluate mixer performance (Clark et al., 2007; Stark and Saensukjaroenphon, 2017). Although the measurement of the chloride ion concentration is commonly accepted by feed manufacturers and nutritionists as a uniformity test, poultry diets amended with L-Lysine HCL (McCoy et al., 1994; Clark et al., 2007) have produced inconsistent results. Therefore, the implementation and exploration of alternative and cost-effective analytical techniques are crucial to monitor feed quality parameters and improve feed formulation adjustments.

Near infrared (**NIR**) reflectance can be defined as an advantageous methodology for the analysis and qualitative evaluation of animal feeds. Previous research has reported the valuable applications of NIR spectroscopy equipment to determine particle size (Williams and Starkey, 1980; Pasikatan et al., 2001; Ely et al., 2008), non-invasive measurements (Givens et al., 1997; Nielsen et al., 2001; Khaleduzzaman et al., 2017), physical and chemical characteristics of raw materials (Nielsen et al., 2001; Smith et al., 2001; Owens et al., 2009), predict adulteration of oils in finished animal feeds (Graham et al., 2012), and quality control in feed conveying systems (Nielsen et al., 2001). The use of NIR spectroscopy for in-line mixer performance determination could be an alternative to continuous quantitative monitoring in the CV% of selected markers in

broiler diets. The evaluation of the interactive effects of mix time, batch size, and methodologies for testing mixer performance including In-line NIR equipment are crucial to optimize mix uniformity (as determined by CV) in broiler diets. Therefore, an experiment was conducted to determine the effects of mix times (4.5 and 0.5 min), batch size (908 and 1815 kg), and methodology to evaluate mixer performance (sodium chloride, Microtracers (Red#40 and Blue#40), and In-line NIR) on mix uniformity of broiler starter diets.

MATERIALS AND METHODS

Feed Formulation, Manufacture, and Experimental Design

Dietary treatments were formulated with corn and soybean meal (SBM) as the primary ingredients (Table 2.1). Twelve batches of broiler starter feed were mixed for 4.5 min (3 min dry mix and 1.5 min of wet mix) and 0.5 min (0 min dry mix and 0.5 min wet mix) to obtain a uniform and a non-uniform mix, respectively. The experiment constituted a 2 × 2 × 4 factorial arrangement of 2 mix times, (4.5 and 0.5 min), 2 batch sizes (908 and 1815 kg), and 4 methodologies to evaluate mixer performance (sodium chloride, Microtracers (Red #40 and Blue #40), and In-line NIR). To create the experimental treatments, dry ingredients were blended in a double shaft counterpoise ribbon mixer with an 1815-kg full load capacity, inlet for major and minor ingredients, a top access door for additional ingredients (hand-adds), and discharge gates (Model TRDB126060, Hayes and Stolz, Forth Worth, TX). All fat in the experimental diets was in the form of poultry oil (1%). In diets mixed for 4.5 min, the dry mix time began after all major, minor, and hand-add dry ingredients were added at the instant the mixer began to run. After the completion of the dry mix cycle, the wet mix cycle began and continued while poultry oil was sprayed into the mixer. In diets mixed for 0.5 min, the mixer was manually controlled, and the mix time was determined based on the amount of time required to add poultry oil into the

batch of feed (0.5 min). The major and minor ingredients (corn, SBM, and poultry by product meal) were discharged into the mixer from the major batch scale. All micro ingredients were individually preweighed into a barrel and added to the mixer at the top access door to prevent a complete dry mix cycle (0 min). For both mix uniformities, the mixing time started when the last ingredient was added to the mixer and ended with mixer discharge, hence it does not include discharge time. Discharge time of the major and micro scales was approximately 0.6 and 0.5 min, respectively. The experimental diets were manufactured in mash form to prevent additional mixing through pelleting and conveying feed systems.

After mixing, the mixer was stopped, the discharge gate was opened, and the mixed feed was conveyed by a drag conveyor (4.3 m), elevated (3.7) m, and dropped into a folding bulk container (908-kg full load capacity). Ten feed samples (0.45 kg of each) were collected from the discharge end using a sampling probe and then divided into smaller aliquots for laboratory analysis. Mash samples were collected at equally spaced time intervals (approximately 0.5 min and 0.25 min/sample) according to previously determined mixer discharge time. Mixed feed discharge time was 5 min/1815 kg and 2.5 min/908 kg batch size of feed. All diets were packaged continuously, and samples were not split to prevent potential further mixing upon discharge, storage, and laboratory analysis. To analyze mix uniformity, representative subsamples of both mix times were analyzed, and mixer CV was determined with the use of a "marker" or nutrient. The inclusion rate of all markers in the dietary treatments was less than or equal to 0.5%.

The chloride ion concentration was determined using the Quantab® Chloride Tritator Procedure (Quantab, Hach Co., Loveland, CO): 1) Samples are ground to achieve a uniform particle size, 2) Weigh 10 g of ground sample, 3) Add 90 g of hot distilled water, stir for 30 s,

wait 60 s, and stir another 30 s, 4) Place a folded filter paper into the cup and then insert a Quantab® strip range 30 to 600 mg/L into the solution, 5) Leave the strip until the yellow peak turns black, 6) Read and convert the strip reading to %NaCl, 7) Multiply the percentage of salt by 10, 8) Calculate the CV (Stark and Saensukjaroenphon, 2017).

The Microtracer count was determined using the Microtracer Rotary Detector Procedure (Red and Blue Count): The rotary detector is a magnetic separator designed to separate Microtracers F from animal diets. 1) Place a 70-mm circle of #1 Whatman filter paper on the rotary magnet, 2) Turn the rotary ON to isolate the Microtracer(s) F, 3) Transfer the magnetic material to a 30 ml analytical scoop using a fantail brush, 4) "Demagnetize" the material with a bulk tape eraser and then sprinkle the material to a 150-185 mm #1 Whatman filter paper, 5) Sprinkle/spread the demagnetized particles as uniformly as possible, 6)Wet the filter paper with 75% ethanol, 7) When spots begin to visually appear in the filter paper, place the filter paper into a preheated oven (149°C) or hot plate, 9) Leave the filter paper until dry, 10) Count the colored spots by circling the spots (Hand count) or download the Microtracers® App to use the spot counter function. 11) Calculate the CV for each set of samples using Poisson statistics and chi-squared calculations.

Whole corn was ground in a hammermill (Model 1522, Roskamp Champion, Waterloo, IA) equipped with 2.4-mm and 3.2 mm-screens to achieve an average particle size of 779 µm. All nutrients and markers selected to determine mixer CV were analyzed for particle size analysis: sodium chloride (391 µm), Microtracers RedF #40 (203 µm), and Microtracers BlueF #40 (209 µm) (Microtracers Inc., San Francisco, CA) (Figure 1). Particle size was determined using a 15-sieve stack with rubber balls, bristle sieve cleaners, and with US sieve numbers 4, 6, 8, 12, 16, 20, 30, 40, 50, 70, 100, 140, 200, 270, and pan. A Ro-Tap shaker (Model RX-29 W.S.

Tyler's Ro-Tap®, Mentor, OH) was used to sift 100 ± 5 g samples for 10 min. Before sifting the sample, 0.5 g of flow agent (Model SSA-58 Gilson's Inc. Sieving Aid, Lewis Center, OH) was added. Geometric mean particle size by mass (Dgw) and the geometric standard deviation of particle diameter by mass (Sgw) were determined using the quantity of material retained on each sieve following the ASABE method S319.4 (ASABE, 2009).

In-line NIR

In the case of the NIR equipment, a Matrix F (Bruker© Optics, Billerica, MA) in line instrument was inserted into a manufacturing stream to collect spectra readings for further analysis. The Matrix F is a FT-NIR that uses fiber optics and connects to a probe (Q412 reflection probe) which is then inserted to a conveyor. Fiber-coupled probes allow spectra collection in real time. The fiber optic probes are connected to the spectrometer using standard SMA connectors or quick connectors. For data collection, the Q412 was welded to a plate that was welded to the conveyor (4.3 m). The sensor head of the probe was mounted to the conveyor window for the continuous analysis of finished feed. It was crucial to have a good flow of feed over the complete window for accurate readings. Individual batches of feed (3 batches/ 4.5 and 0.5 mix times, and 3 batches/908 and 1815 kg) were discharged into the surge conveyor and samples were automatically removed from the line, sent to the NIR and a computer for analyses. Data were analyzed using a vibrational spectroscopy Optics User software (OPUS Software, São Paulo, Brazil) which uses Partial Least Squares (**PLS**) regression calibration models. A total of 465 readings were obtained from the proximate analysis (ash, fat, fiber, moisture, and protein) throughout the manufacturing of feed at two mix times (4.5 and 0.5 min) and two batch sizes (908 and 1815 kg). Although, proximate analyses were obtained for each batch of feed, only protein values were selected for mixer CV calculations.

Measurements

Coefficient of variation. Subsamples of mash feed were analyzed for sodium chloride, Microtracers RedF #40, and Microtracers BlueF #40, and a coefficient of variation (CV) was calculated for each of the mixing uniformities. The industry standard for CV to obtain a uniform mix is less than 10%. The coefficient of variation is calculated using the following formula:

Mean (μ) = $\sum$ xi / n

Standard Deviation (s)= $\sqrt{\sum (xi - \mu)2 / (n-1)}$

Percent Coefficient of Variation (% CV) = (s / μ) * 100

Conformity Index (CI). A conformity method tests whether a sample "conforms" to defined reference material within a certain threshold. Conformity models are built from a collection of "reference samples," which are samples defined as "good". These samples meet the criteria to essentially be a control sample that would pass Quality Assurance/Quality Control. Therefore, these samples are representative of the final product (a pass). Additionally, natural variation in the sample and process should be considered for the reference spectra. In the present study, a conformity model was built using the data from the 4.5 min total mix time and a batch size of 1815 kg as reference spectra. An average was calculated at each absorbance value for all of the spectra which yields a CI, and this produces a conformity threshold. In quantitative terms, an average spectrum is generated, which is represented graphically by a red line that falls in the middle of two additional red-colored lines (boundaries), which are the thresholds for conformity. Anything that falls within those lines "conforms" and anything outside "fails" the conformity test.

$$\text{Conformity Index (CI)} = \frac{\text{Absorbance of a sample} - \text{Absorbance of the average}}{\text{Standard Deviation}}$$

Statistical Analysis

In the present experiment, results were analyzed as a 2 × 2 × 4 factorial (mix time × batch size × methodologies to evaluate mixer performance) complete randomized design. Data were analyzed using the GLM procedure of JMP software (JMP, 2010) with the following mixed-effects model:

$$Yi\,j = \mu + \rho i + \tau\, j + \varepsilon i\, j$$

Where *Yij* = observed response on pellets; μ = is the overall mean; the ρi are identically and independently normally distributed random effects with mean 0 and variance σ2ρ; the $\tau\, j$ are fixed factor level effects corresponding to the jth dietary treatment (treatments 1 to 16) such that $\tau\, j = 0$; and the εij are identically and independently normally distributed random errors with mean 0 and a variance σ2. The mean values among the 16 treatments were compared using the Tukey's honestly significant different procedure with statistical significance considered at $P \leq 0.05$.

RESULTS

The main effects of mix times, batch sizes, and mixer performance methodologies on CV are reported in Table 2.2. A total mixing time of 4.5 min and samples analyzed with the In-line NIR generated lower mixer CVs compared to 0.5 min, and the Microtracers and sodium chloride methods ($P \leq 0.01$). However, there were no significant differences between batch sizes on mixer CV.

The interaction effects of mix times, batch sizes, and mixer performance methodologies on mixer CV are reported in Table 2.2. There were no significant interactions of mix times, batch sizes, and mixer performance methodologies on mixer CV ($P \geq 0.05$). However, interactions between mix times and methodologies observed in this experiment provide a better

understanding of how a longer mix time improves nutrient homogeneity as shown by the different markers selected being distributed uniformly across the batch yielding a lower mixer CV ($P \leq 0.01$). The highest mixer CV was observed on diets mixed for 0.5 min and analyzed with Microtracer Blue #40. In contrast, mixer CV decreased on diets mixed for 4.5 min while analyzed with sodium chloride and Microtracers (Red and Blue #40). Even though the In-line NIR generated the lowest mixer CV, there were no significant differences between the two mixing times. Moreover, similarities were observed on sodium chloride mixer CVs regardless of mixing time.

DISCUSSION

In agreement with the main effects observed for mix time, a longer mix time decreases mixer CV% because there is a uniform blend of the markers analyzed throughout the batch of feed. Although increased mixing time has been reported to increase mix uniformity in broiler diets (Pfost et al., 1974; McEllhiney and Olentine, 1982; Wilcox and Balding 1986; Wicker and Poole, 1991; McCoy et al., 1994), batch size or mixer fill may also have an impact in mixer performance. In agreement, Wicker and Poole (1991) reported that increasing mix time is not sufficient to correct mixing issues caused by overfilling of the mixer. In contrast, Martin (2005) concluded that underfilling the mixer could interrupt the flow of ingredients within the mixing chamber which can negatively impact mix uniformity. Therefore, the lack of differences between batch sizes could mean that as long as the mixer is not fill or underfill beyond rated capacity, it may not impact the degree of variation in mixed feeds.

The adequate selection of a marker or nutrient and type of assay performed are critical to monitor mixer performance in broiler diets. However, differences in sampling method (Reese et al., 2017), accuracy, and costs have an impact in the decision-making process of marker selection

and methodology used for determining mix uniformity (Clark et al., 2007). In contrast to the consistency between the sodium chloride and Microtracer's mixer CVs observed, McCoy et al. (1994) reported a decrease in CV for sodium chloride concentrations compared with Microtacers when mixing time increased in preliminary mixer evaluations. However, the authors reported no statistical differences on mixer CV when experimental diets were mixed at 20 and 80 mixer revolutions and analyzed for salt and sodium using the Quantab® and Omnion® methods, respectively. Similarly, Ciftci and Ercan (2003) reported that mixer CVs of sodium chloride decreased from 39.89% (0.20 min) to 13.85% (0.59 min) to 7.95% (3.75 min) using the Merckquant chloride test compared to the titration method which reported no statistical differences at 0.59 and 3.75 min total mix time. A later study reported numerical reductions on sodium chloride and Microtracer Red #40 (absorbance method) CVs when diets were mixed for 0.5, 2.5, and 5.0 min but only the Microtracer Red #40 (hand-count method) and Microtracer RF-Blue Lake (qualitative method) reported statistical differences (Clark et al., 2007). The mixer CV of the Microtracer Red #40 and RF-Blue Lake decreased from 21.77% to 10.43% and from 32.49% to 18.64% when mix time increased from 0.5 to 5.0 min, respectively. Moreover, Zawislak et al. (2011) analyzed mineral mixtures with Microtracer Blue #40 and reported a decrease of 12% in CV when mix time increased from 5 to 8 min but no further decreases at 10 min. In the McCoy et al. (1994), Ciftci and Ercan (2003), and Clark et al. (2007) studies, the experimental diets were amended with L-Lysine HCL which may alter the chloride ion concentration (Clark et al., 2007), nonetheless broiler diets in the present study were amended with L-Lysine HCL and choline chloride to replicate diet composition commonly fed to broilers in the industry. This could explain why the CVs of sodium chloride were similar at 4.5 and 0.5 min. In the case of the Microtracers, the use of quantitative vs. qualitative methods could have an

impact on the interpretation of uniformity in mixed feeds. Although previous research tested up to 11 different markers simultaneously and reported CVs that ranged from 6 % to 54 % (McCoy et al., 1994; Clark et al., 2007), the CVs observed with sodium chloride and Microtracers (Red and Blue #40) ranged from 8% to 18 % which indicates that these methods provided a degree of variation similar to the standards set by the industry. The different markers and methods selected for testing mixer uniformity have an impact on CV and assay costs. Therefore, efforts should be made to maintain optimal mixing times, select single source ingredients, monitor assay methodologies, and thus mixer performance to ensure nutrient homogeneity is optimized.

The In-line NIR prove to be a practical tool to determine proximate analysis in real time. However, the mixer CVs generated by the protein values from the proximate analysis were similar at 4.5 and 0.5 min total mix time. Near-Infrared reflectance is sensitive to physico-chemical characteristics found in raw materials and cereal grains (Pasikatan et al., 2001). In addition, although the present study was designed to evaluate the effects of methodologies on mixer performance, previous research has reported that particle size and variation in nutrient profile of ingredients have an impact on the chemical composition of feed analyzed by NIR equipment (Mel-cion 1974; Norris and Barnes, 1976; Pedamond, 1977; Williams and Starkey, 1980; Nathier-Dufour et al., 1995). Since the readings of the NIR are affected by the geometry of the particles, the particle size effects on spectra may be considered “noise”, thus the samples collected must be uniformly ground (Wendtlandt and Hecht, 1966; Pasikatan et al., 2001). The particle size of corn used in the present study was 779 µm to maintain a continuous flow of feed as it was conveyed and detected by the sensor head of the probe. Moreover, Creger (1957) reported that the higher density of sodium chloride made the particles to “sift” the carrier (SBM) while the lower density of particles in nitrophenide “adhere” to the particles of SBM; hence

explaining the improved mixing capabilities of sodium chloride with the carrier in a shorter period of time. The authors reported a similar effect when sodium chloride and nitrophenide were mixed with corn ground through a 1/8 inch screen and concluded that the particle size distribution of markers play a role in mix uniformity. The geometric mean diameter of the markers used are shown in Figure 2.1. Even though both Microtracers had similar particle size (203 and 209 µm), the sodium chloride used in this experiment was slightly coarser (391 µm) and followed a different particle size distribution. In addition, this could explain the lower mixer CV% obtained with sodium chloride regardless of mix time. Therefore, in the case of NIR technology, the overall particle size of the feed samples collected by the In-line NIR were not uniform across all batches. Furthermore, the number of readings obtained with an In-line NIR depend on the amount of feed flowing through the welded window at the conveyor. Mateo-Ortiz et al. (2014) reported that probe location or position and paddle wheel speed have an impact on NIR predictions in a tablet press. A batch size of 1815 kg allows more time for data collection by the equipment compared to a batch size of 908 kg which flows at a faster speed and may not cover the window uniformly for the probe light beam.

In the present study, it is critical to acknowledge the importance of single source markers, the response on mixer CV was not as expected from the protein values obtained from the experimental diets. Previous research has reported that most of the protein analyses are affected by the physical composition of other ingredients in the diet (Williams and Starkey, 1980). However, In-line NIR imaging is a fast and adequate measurement tool for understanding the behavior of spectra in a continuous feed conveying system. Therefore, a conformity test was performed on all twelve batches to have a better understanding of the spectral behavior at two mix times and batch sizes (Figure 2.2). Like mentioned earlier, the reference data utilized for the

conformity model was the long run (4.5 min) with an 1815 kg batch size because it provided the greatest differences when compared to the other batches. In general, the default CI limit is 3, nevertheless specific spectral regions from observations of a previous model were considered, thus a CI limit of 2.8 was used for this model. A CI limit of 2.8 was ideal because the majority of the test spectra "failed" which is expected for different mix times and batch sizes (4.5 min/908 kg, 0.5 min/908 kg, and 0.5 min/1815 kg) differing from the reference (4.5 min/1815 kg). However, out of 63 test samples, 6 "conformed" or passed the test which belong to the test spectra of 1815 kg with a total mix time of 0.5 min. The comparison of both mix times and batch sizes within the conformity test are reported in Figure 2.2. To have a better understanding of the response observed, two datapoints (#33 and #58) were highlighted in yellow in the graph. Although both samples correspond to a total mix time of 0.5 min, sample #33 (1815 kg) has a max CI value of 11.43 while sample #58 (908 kg) correspond to a different batch size and reported a max CI value of 3.20. Therefore, considering both batch sizes, a shorter mix time increases the differences observed on CI values from the reference spectra. Moreover, graphic representations of the raw spectra for the 0.5 min total mix time and the reference spectra are reported in Figure 2.3 and 2.4, respectively. A closer look to the raw spectra collected by the In-line NIR facilitated a visual representation of the differences observed in the spectra readings between runs. However, the addition of "calculations" or "biases" to methods for individual components like protein require further investigation to correct for the issues observed on estimating mixer CV performance with In-line NIR equipment.

CONCLUSIONS AND APPLICATIONS

1. A total mix time of 4.5 min decreased mixer CV by 5% compared to 0.5 min. However, there were no statistical differences on mixer CV between batch sizes.
2. The mixer CVs of sodium chloride (Cl ion) and protein from the proximate analysis of the In-line NIR provided cofounding results. However, both Microtracers (Red and Blue #40) performed like expected to estimate mix uniformity which emphasizes the importance of using a single source as a marker that will not interfere with other ingredients in the diet.
3. Although the markers selected estimated differences on mixer CV performance at two mix times and batch sizes, a further exploration of the conformity test and spectra readings with the In-line NIR provided practical representations of the behavior of samples during mixer discharge of finished feed.

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Table 2.1. Ingredient and nutrient composition of a broiler starter diet.

Ingredient, %	Starter
Corn	58.94
Soybean Meal, 48 % Crude Protein	31.75
Poultry by Product Meal	5.00
Poultry Oil	1.00
Monocalcium phosphate, 21% P	0.90
Calcium carbonate	0.84
Sodium chloride	0.50
D-L Methionine (99%)[1]	0.30
Trace mineral premix[2]	0.20
Choline Chloride	0.20
L-Lysine-HCl (78%)	0.15
L-Threonine	0.10
Vitamin Premix[3]	0.05
Selenium, 0.06%[4]	0.05
Phytase[5]	0.01
Microtracers (Red[6] and Blue[7] #40)	0.01
	100.00
Calculated analysis, % (unless otherwise noted)	
AMEn, kcal/kg	3,000
Crude Protein	22.83
Digestible Lys	1.37
Calcium	1.00
Available phosphorus	0.50

Abbreviation: AME_n, apparent metabolizable energy

[1]Donated by Evonik Corporation

[2]Mineral premix include per kg of diet: Mn (manganese sulfate), 120 mg; Zn (zinc sulfate), 120 mg; Fe (iron sulfate monohydrate), 80 mg; Cu (tri-basic copper chloride), 10 mg; I (ethylenediamine dihydriodide), 2.5 mg; and Co (cobalt), 1 mg.

[3]Donated by DSM Nutritional Products North America. Vitamin premix includes per kg of diet: Vitamin A (Vitamin A acetate), 6600 IU; Vitamin D (cholecalciferol), 1980 IU; Vitamin E (DL-alpha tocopherol acetate), 33 IU; menadione (menadione sodium bisulfate complex), 2 mg; Vitamin B12 (cyanocobalamin), 0.02 mg; folacin (folic acid), 1.1 mg: D-pantothenic acid (calcium pantothenate), 11 mg; riboflavin (riboflavin), 6.6 mg; niacin (niacinamide), 55 mg; thiamin (thiamin mononitrate), 2 mg; D-biotin (biotin), 0.13 mg; and pyridoxine (pyridoxine hydrochloride), 4 mg.

[4]Selenium premix provided Se at 0.3 mg/kg of feed.

[5]Quantum® Blue 5G (Donated by AB Vista Feed Ingredients, Marlborough, UK) provides per kg of diet: 500 FTU/kg of phytase activity.

[6]Microtracer Red #40 Fe marker added at 50g/ton.

[7]Microtracer Blue #40 Fe marker added at 50g/ton.

Table 2.2. Main and interaction effects of mix time, batch size, and methodology on mixer coefficient of variation (CV).

Methodology	Mix time,	Batch size,	CV, %[2]
Main effects			
Chloride ion (as sodium chrloide)			13.99[A]
Microtracer Red #40 (count)[3]			12.53[A]
Microtracer Blue #40 (count)[4]			13.04[A]
In-line NIR			1.49[B]
P-value			<.0001
SEM[1]			0.89
	4.5		7.50[B]
	0.5		13.02[A]
	P-value		<.0001
	SEM		0.63
		908	10.93
		1815	9.60
		P-value	0.1432
		SEM	0.63
Interaction effects			
Chloride ion (as sodium chloride)	4.5	-	11.38[BC]
Microtracer Red #40 (count)	4.5	-	8.72[C]
Microtracer Blue #40 (count)	4.5	-	8.01[C]
In-line NIR	4.5	-	1.90[D]
Chloride ion (as sodium chloride)	0.5	-	16.61[AB]
Microtracer Red #40 (count)	0.5	-	16.35[AB]
Microtracer Blue #40 (count)	0.5	-	18.06[A]
In-line NIR	0.5	-	1.07[D]
P-value			0.0010
SEM			1.25

[A, B]Means within a column with different superscripts differ significantly ($P \leq 0.01$).

[1]SEM =Standard error of the mean for n=330 readings for each main effect of methodology, n=330 for each main effect of pellet mill die L:D, and n=55 readings for each interaction of methodology, mix time, and batch size.

[2]CV= Coefficient of variation (CV = (Standard Deviation/Mean) *100). Mash samples (3 kg of each) were collected at equally spaced intervals using a sampling probe (n=10) and then divided into smaller aliquots for laboratory analysis. Mixed feed discharge time was 5 min/908 kg.

[3]Microtracer Red #40 Fe marker added at 50g/ton.

[4]Microtracer Red #40 Fe marker added at 50g/ton.

[5]The broiler starter diets were batched as to utilize half or full capacity of a double shaft counterpoise ribbon mixer on a weight basis 908 and 1815 kg/batch, respectively.

[6]In the 4.5 min mix time diets, after the completion of the dry cycle, the wet cycle began and continued while the poultry fat was sprayed into the mixer. In the 0.5 min mix time diets, the mixer was manually controlled, and the amount of time required was based on the addition of poultry fat into the batch of feed (0.5 min). All micro ingredients were individually pre weighed and added at the top access door to prevent a complete mix. Therefore, dry and wet cycles were done simultaneously while the major and minor ingredients discharged. Discharge time of the major and micro scales were approximately 0.6 and 0.5 min, respectively.

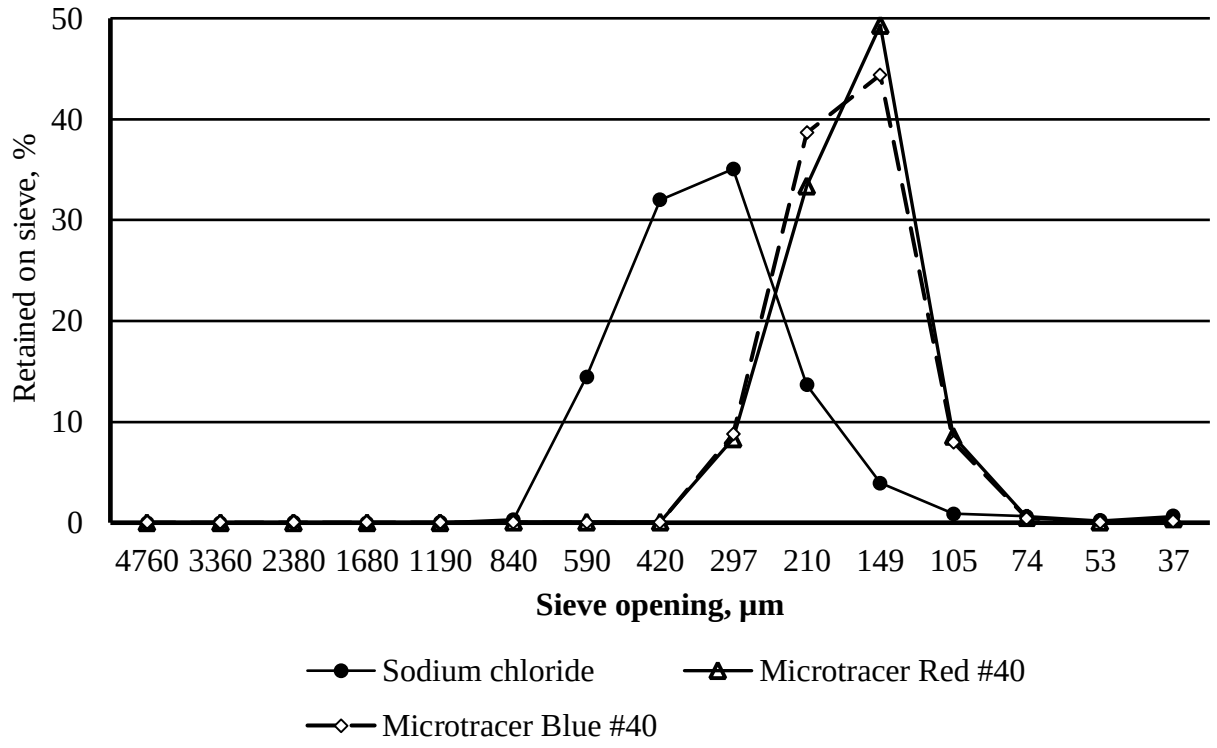

Figure 2.1. The geometric mean diameter by mass (Dgw) and particle size distribution of markers and nutrients before mixing was determined. The inclusion rate of all markers in the dietary treatments was less than or equal to 0.5%. Twelve batches of broiler starter feed were mixed for 4.5 min (3 min dry mix and 1.5 min of wet mix) and 0.5 min (0 min dry mix and 0.5 min wet mix) to obtain a uniform and a non-uniform mix, respectively. The experiment constituted a 2 × 2 × 4 factorial arrangement of 2 mix times, (4.5 and 0.5 min), 2 batch sizes (908 and 1815 kg), and 4 methodologies to evaluate mixer performance (sodium chloride, Microtracers (Red#40 and Blue#40), and In-line NIR). To create the experimental treatments, dry ingredients were blended in a double shaft counterpoise ribbon mixer with an 1815-kg full load capacity, inlet for major and minor ingredients, a top access door for additional ingredients (hand-adds), and discharge gates. All fat in the experimental diets was in the form of poultry oil (1%). All nutrients and markers selected to determine mixer CV were analyzed for particle size analysis: sodium chloride (391 µm), Microtracers Red #40 (203 µm), and Microtracers Blue #40 (209 µm).

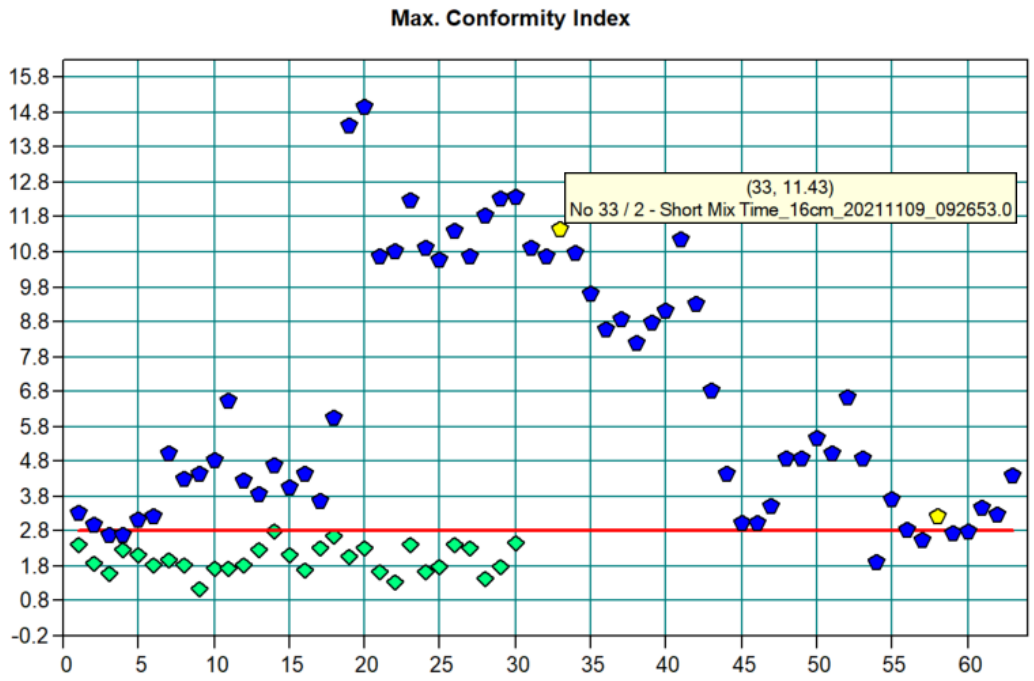

Figure 2.2. Graphic representation of a conformity test for the 4.5 and 0.5 min mix times and 908 and 1815 kg batch sizes. The inclusion rate of all markers in the dietary treatments was less than or equal to 0.5%. Twelve batches of broiler starter feed were mixed for 4.5 min (3 min dry mix and 1.5 min of wet mix) and 0.5 min (0 min dry mix and 0.5 min wet mix) to obtain a uniform and a non-uniform mix, respectively. The experiment constituted a 2 × 2 × 4 factorial arrangement of 2 mix times, (4.5 and 0.5 min), 2 batch sizes (908 and 1815 kg), and 4 methodologies to evaluate mixer performance (sodium chloride, Microtracers (Red#40 and Blue#40), and In-line NIR). To create the experimental treatments, dry ingredients were blended in a double shaft counterpoise ribbon mixer with an 1815-kg full load capacity, inlet for major and minor ingredients, a top access door for additional ingredients (hand-adds), and discharge gates. All fat in the experimental diets was in the form of poultry oil (1%). To build the conformity model, only 2 mix time and batch size were considered. A conformity index (CI) of 2.8 was determined (Red Line). The reference spectra is indicated in Green and the test spectra in Blue. The yellow points correspond to sample #33 and #58, both with a 0.5 min total mix time but 1815 and 908 kg batch sizes, respectively.

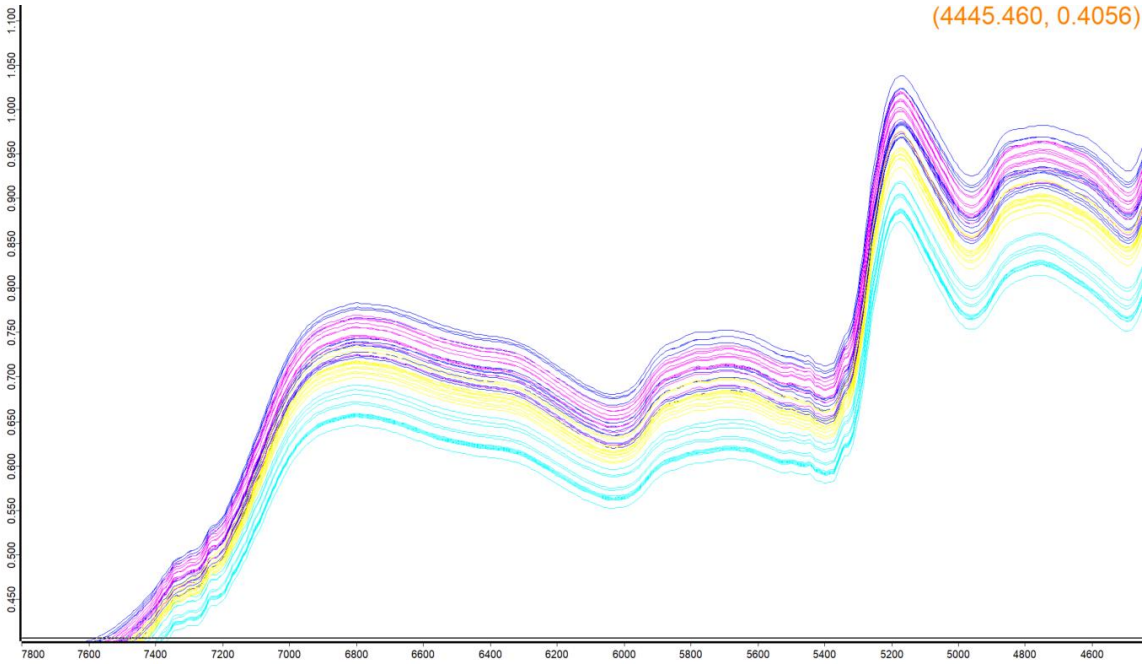

Figure 2.3. Graphic representation of the Raw Spectra (no preprocessing) for the 0.5 min total mix time. The inclusion rate of all markers in the dietary treatments was less than or equal to 0.5%. Twelve batches of broiler starter feed were mixed for 4.5 min (3 min dry mix and 1.5 min of wet mix) and 0.5 min (0 min dry mix and 0.5 min wet mix) to obtain a uniform and a non-uniform mix, respectively. The experiment constituted a 2 × 2 × 4 factorial arrangement of 2 mix times, (4.5 and 0.5 min), 2 batch sizes (908 and 1815 kg), and 4 methodologies to evaluate mixer performance (sodium chloride, Microtracers (Red#40 and Blue#40), and In-line NIR). To create the experimental treatments, dry ingredients were blended in a double shaft counterpoise ribbon mixer with an 1815-kg full load capacity, inlet for major and minor ingredients, a top access door for additional ingredients (hand-adds), and discharge gates. All fat in the experimental diets was in the form of poultry oil (1%). The Dark Blue spectra represents all of the runs for 0.5 min mix time and 908 kg batch size. However, the runs for 0.5 min and 1815 kg batches were separated: Bright Blue (run #7), Magenta (run #8), and Yellow is run (#12).

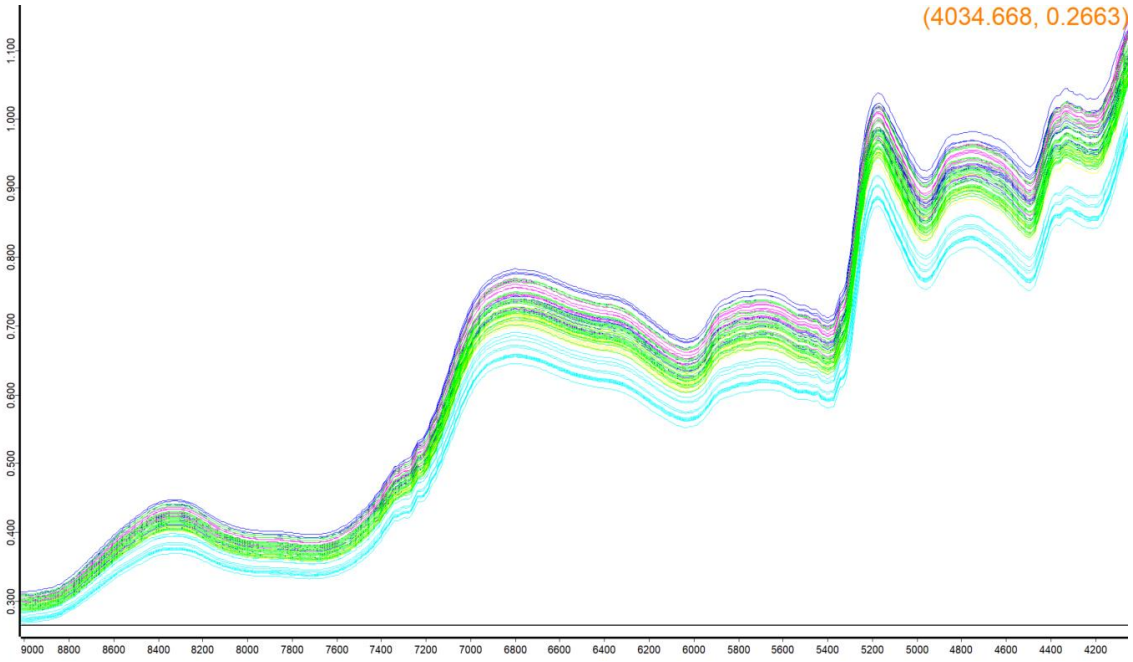

Figure 2.4. Graphic representation of the Raw Spectra (no preprocessing) for the reference spectra (4.5 min total mix time and 1815 kg), 4.5 min-908 kg, and 0.5 min- 908 and 1815 kg. The inclusion rate of all markers in the dietary treatments was less than or equal to 0.5%. Twelve batches of broiler starter feed were mixed for 4.5 min (3 min dry mix and 1.5 min of wet mix) and 0.5 min (0 min dry mix and 0.5 min wet mix) to obtain a uniform and a non-uniform mix, respectively. The experiment constituted a 2 × 2 × 4 factorial arrangement of 2 mix times, (4.5 and 0.5 min), 2 batch sizes (908 and 1815 kg), and 4 methodologies to evaluate mixer performance (sodium chloride, Microtracers (Red#40 and Blue#40), and In-line NIR). To create the experimental treatments, dry ingredients were blended in a double shaft counterpoise ribbon mixer with an 1815-kg full load capacity, inlet for major and minor ingredients, a top access door for additional ingredients (hand-adds), and discharge gates. All fat in the experimental diets was in the form of poultry oil (1%). The reference spectra (4.5 min and 1815 kg) in Green with all of the spectra for the rest of the runs.

CHAPTER 3

Comparison between an in-line implementation of a temperature profile probe-based tool vs. three non-contact methodologies to monitor hot pellet temperature in swine and broiler diets

SUMMARY

Previous research has demonstrated that the heat generated during the pelleting process has an impact on thermo-sensitive nutrients. Even though conditioning temperatures are obtained in real-time, the frictional heat generated by the die can only be estimated using non-contact methodologies. Therefore, the objective of these experiments was to evaluate the current available methodologies compared to an in-line methodology to measure hot pellet temperature (**HPT**). Experiment 1 consisted of a swine lactation diet conditioned at three conditioning temperatures (74°, 79°, and 85° C). Experiment 2 consisted of a broiler starter diet with 7 treatments, varying moisture contents from mixer added water (0, 1, and 2%) and conditioner added steam (0, 2, 4%). A mash diet not subjected to mixer added water nor conditioner added steam was dry pelleted to serve as negative control (**NC**). In experiment 1 and 2, three non-contact methodologies were used to collect HPT readings at die exit: Insulated thermos, Styrofoam bucket, and Infrared (**IR**) gun. Experiment 3 constituted a 2×2×3 factorial arrangement of 2 fat levels (1 and 4%), 2 die L:D ratios (6.5 and 10), and 3 conditioning temperatures (74°, 79°, and 85° C). A temperature profile probe-based tool was used to collect HPT at die surface. In experiment 1 and 2, the lowest HPT was obtained when the sample was analyzed with the IR gun ($P < 0.01$). In experiment 2, interactions were apparent ($P < 0.01$) for all treatments. The results of these experiments indicated that HPT readings differed depending on the methodology used.

DESCRIPTION OF PROBLEM

During the conditioning and pelleting processes, mash feed is subjected to heat, pressure, and moisture which may result in the denaturation of exogenous enzymes and impact the stability of thermo-sensitive nutrients in feed. Although pelleting at high temperatures can influence the thermal treatment of additives and ingredients, commercial facilities still commonly choose to pellet at temperatures ≥ 95 °C to control salmonella, feed hygiene, and pellet quality (Veldman et al., 1995; Jones and Richardson, 2004). However, the frictional heat generated by the pellet mill die (Skoch et al., 1981; Cutlip et al., 2008; Loar II and Corzo, 2011; Fahrenholz, 2012) is often isolated from the decision-making process, which typically means that the temperature of extruded pellets is higher than that of the mash exiting the conditioner.

Although previous research has evaluated the effects of conditioning temperature on thermo-sensitive additives in feed (Inborr and Bedford, 1994; Spring et al., 1996; Silversides and Bedford, 1999; Jensen, 2000; Amerah et al., 2011; Abdollahi et al., 2013; Pope and Fahrenholz, 2020), the impact of the pellet mill die, which is the primary point of extrusion and alters the physicochemical characteristics in feed, remains as a less-understood part of the pelleting process. Non-contact methods (infrared technology or insulated containers) are currently used to record hot pellet temperature readings at the exit of the pelleting chamber, after some cooling has likely already occurred (Salas-Bringas et al., 2007). However, the die rotational speed (Fairfield et al., 2005), presence of steam (Salas-Bringas et al., 2007), and the cost and complexity (Wang et al., 2006) of manufacturing and installing the ideal wireless sensor without interfering with production makes the monitoring of pellet temperatures in real time a significant challenge. Moreover, research evaluating a contact method or device to simultaneously record the temperature of pellets as they are extruded from the pellet die while operating the pellet mill is

sparse. Therefore, a study was conducted to evaluate the current available non-contact methodologies compared to a novel in-line methodology to estimate the rise in hot pellet temperature (**HPT**) as they are extruded by the die.

MATERIALS AND METHODS

Feed formulation, Manufacture, and Experimental Design

In experiment 1 and 2, a swine lactation and a broiler starter diet were manufactured to evaluate the current available non-contact methodologies on HPT (Table 3.1 and 3.2). In experiment 3, a broiler starter was manufactured to evaluate an in-line methodology on HPT. In all three experiments, treatments were formulated with corn and soybean meal (SBM) as the primary ingredients. Dry ingredients were blended in a double shaft counterpoise ribbon mixer with a 1815-kg full load capacity, inlet for major and minor ingredients, a top access door for additional ingredients (hand-adds), and discharge gates (Model TRDB126060, Hayes and Stolz, Forth Worth, TX). In experiment 1, three batches (454 kg/batch) of feed were mixed for 4.5 min (3 min dry mix and 1.5 min of wet mix) to obtain a uniform mix. Diets were conditioned at three conditioning temperatures (74°, 79°, and 85° C) for 30 s in a single pass conditioner (Model C18LL4/F6, California Pellet Mill Co., Crawfordsville, IN). In experiment 2 and 3, three batches (454, 680, and 907 kg/batch) and one batch (454 kg/batch) (experiment 2), one batch (454 kg/batch) (experiment 3) of feed were mixed for 4.5 min (3 min dry mix and 1.5 min of wet mix) to obtain a uniform mix, respectively. In both experiments, mash diets were subjected to a conditioner retention time for 30 s in a single pass conditioner (Model C18LL4/F6, California Pellet Mill Co., Crawfordsville, IN) and were conditioned at one, two, and three conditioning temperatures depending on batch size (experiment 2) and at three conditioning temperatures (experiment 3).

Experiment 2 consisted of a broiler starter diet with 7 treatments, varying moisture contents from mixer added water (**MW**) (0, 1, and 2%) and conditioner added steam (**CS**) (0, 2, 4%): 0% MW, 2% CS (Trt 1), 1% MW, 1% CS (Trt 2), 2% MW, 0 % CS (Trt3), and 2% MW, 2% CS (Trt 4). A mash diet not subjected to mixer added water nor conditioner added steam was dry pelleted to serve as negative control (**NC**). For batches amended with steam, conditioning temperature was increased by -3.89 °C (25 °F) for every 1% of target conditioner added steam (Chuah et al., unpublished). A treatment subjected to 0% mixer added water, but 4% steam served as a positive control (**PC**) to replicate the standard pelleting conditions in the industry. A treatment subjected to 4% mixer added water and 0% conditioner added steam served as a second positive control (**PC2**) (Chuah et al., unpublished). Experiment 3 constituted a 2 × 2 × 3 factorial arrangement of 2 fat inclusion levels (1% and 4%), 2 die L:D ratios (6.5 and 10), and 3 conditioning temperatures (74°, 79°, and 89° C).

Broiler starter and swine lactation diets were pelleted using a 30 HP pellet mill (907 kg/hr) (Model PM 1112-2, California Pellet Mill Co., Crawfordsville, IN) equipped with a 4.4 × 29 mm die (experiment 1, 2 and 3) and 4.4 × 45 mm die (experiment 3). All experimental runs were performed using a warm die. Pellets were cooled with ambient air in a counter-flow pellet cooler (Model VK09X09KL, Geelen Counterflow USA Inc., Orlando, FL). In experiment 1 and 2, three non-contact methodologies were used to determine HPT at die exit: 1) Insulated thermos (0.13 gal), 2) Styrofoam bucket (5 gal), and 3) Infrared (**IR**) gun (handheld). A thermometer was inserted into the thermos and bucket to measure HPT. A total of 91 and 189 HPT readings were collected for experiment 1 and 2, respectively. In experiment 3, a temperature profile probe with 8 sensing points located within the pelleting chamber was used to collect 660 HPT readings at the die surface in real time.

In all three experiments, whole corn was ground in a hammer mill (Model 1522, Roskamp Champion, Waterloo, IA) equipped with 2.4-mm and 3.2 mm-screens to achieve an average particle size of 332 µm. Particle size was determined using a 15-sieve stack with rubber balls, bristle sieve cleaners, and with US sieve numbers 4, 6, 8, 12, 16, 20, 30, 40, 50, 70, 100, 140, 200, 270, and pan. A Ro-Tap shaker (Model RX-29 W.S. Tyler's Ro-Tap®, Mentor, OH) was used to sift 100 ± 5 g samples for 10 min. Before sifting the sample, 0.5 g of flow agent (Model SSA-58 Gilson's Inc. Sieving Aid, Lewis Center, OH) was added. Geometric mean particle size by mass (Dgw) and the geometric standard deviation of particle diameter by mass (Sgw) were determined using the quantity of material retained on each sieve following the ASABE method S319.4 (ASABE, 2009).

Measurements

Hot pellet temperature. In all three experiments, subsamples of pelleted feed were collected for HPT. In the case of the non-contact methods (experiment 1 and 2), pellets were collected at die exit, placed in the container (insulated thermos and styrofoam bucket), and temperature was measure by inserting a digital thermometer. The highest reading once the temperature reached equilibrium was recorded for further analysis. The handheld IR gun was pointed directly to pellets at die exit. In all three experiments, temperature measurements were taken prior to and immediately after the pellet die once the batch was operating at steady state.

In experiment 3, a temperature profile probe with 8 sensing points located within the pelleting chamber was used to collect HPT readings at the die surface in real time. A custom-made 8 point profile probe type J thermocouple (OSK2K4334 OmegaTM profile probes, Norwalk, CT) was mounted to a pellet mill knife (California Pellet Mill Co., Crawfordsville, IN). The probe was mainly composed of a 1.59 × 8.89 cm transitioning fitting attached to a 19.05 cm

(length) stainless steel thermocouple with a grounded junction. Each sensing point had a distance of 1.27 cm and a tolerance of continuous temperatures up to 260° C. Moreover, the transitioning fitting of the probe was attached to a 101.6-cm PFA lead wire terminating in 8 miniature connectors (one for each sensing point). The mini flat male terminating miniature connectors with integral cable clamp caps were removed and adapted to fit a data logger. Readings were then recorded by the data logger (Model OM-CP-OCTPRO Omega^{TM} portable data acquisition logger, Norwalk, CT) while running the pellet mill and exported to a computer for further analysis. The probe was able to collect a total of 9,216 HPT readings (2 min/trt). Despite the large number of readings collected, sensing point #8 registered the highest readings (closest to the pellet mill die) hence, these were the HPTs used for the statistical analysis.

Die retention time. In experiment 3, the effects of two L:D ratios were evaluated on HPT (Table 3.3 and 3.4). Therefore, die retention time was calculated for both L:D ratios using the formula proposed by Saensukjaroenphon et al. (2019):

Die retention time (s) = Amount of material in the effective length of the die (g) / Mass flow rate (g / s)

The internal die surface area (cm^2), number of holes per cm^2, effective volume per hole (cm^3), material density (g/cm^3) were multiplied to calculate the amount of material in the effective length of the die (g).

Statistical Analyses

In the experiment 1 and 2, data were analyzed as a one-way ANOVA using the GLM procedure of JMP software (JMP, 2010) with the following model:

$$Yi\,j = \mu + Ti + \varepsilon ij$$

Where $Yi\,j$ = the observed response on pellets; μ = is the overall mean; Ti = fixed effect of treatment; and εij = residual error, εij N(0, σ2ε). The mean values among treatments were

compared using the Tukey's honestly significant different procedure with the significant level at $P \leq 0.05$.

In experiment 3, results were analyzed as a 2 × 2 × 3 factorial (fat inclusion level × L:D ratio × conditioning temperatures) complete randomized design. Data were analyzed using the GLM procedure of JMP software (JMP, 2010) with the following mixed-effects model:

$$Yi\,j = \mu + \rho i + \tau\, j + \varepsilon i\, j$$

Where *Yij* = observed response on pellets; μ = is the overall mean; the ρi are identically and independently normally distributed random effects with mean 0 and variance σ2ρ; the τ j are fixed factor level effects corresponding to the jth dietary treatment (treatments 1 to 12) such that $\tau\, j = 0$; and the εij are identically and independently normally distributed random errors with mean 0 and a variance σ2. The mean values among the 12 treatments were compared using the Tukey's honestly significant different procedure with statistical significance considered at $P \leq 0.05$.

RESULTS AND DISCUSSION

Non-contact methodologies

Hot pellet temperature data for experiment 1 and 2 are reported in Figure 3.1 and 3.2, respectively. Although both studies have different ingredient and nutrient composition, and manufacturing conditions, the three current available non-contact methodologies commonly used by the industry were evaluated. In experiment 1, a lower HPT was obtained when the sample was analyzed with the IR gun compared with samples analyzed with the insulated thermos and the styrofoam bucket at 79° C conditioning temperature ($P \leq 0.01$) (Figure 3.2). Previous research has reported that an increase in steam addition at the conditioner increases HPT (Moritz et al., 2003; Lilly et al., 2011; Boney and Moritz et al., 2017). However, there were no statistical

differences on HPT readings when mash diets were conditioned at 74° C and 85° C regardless of the methodology used ($P \leq 0.01$) (Figure 3.2). A swine lactation diet with 4% inclusion level of poultry oil was evaluated. Fat is one of the few feedstuff materials that have consistent results on pellet quality and when the inclusion is above 2%, the resulting die lubrication (Richardson and Day, 1976; Salmon, 1985) may decrease HPT readings. However, a fat inclusion of 4% is uncommon for poultry diets due to high prices, detrimental effects on the physical integrity of pellets, and increased amount of fines (Hancock, 2010; Gehring et al., 2011). As an alternative to reduce poor pellet quality, researchers have evaluated the effects of fat application at the mixer and its impact on die lubrication. Corey et al. (2014) reported that diets with 3% mixer-added fat decreased HPT compared to diets with 1 % mixer-added fat at a conditioning temperature of 82°C in broiler finisher diets. The authors stated that treatments containing 1% mixer-added fat had an additional 2% fat inclusion after pelleting while treatments with 3% mixer-added fat had no additional fat added. Moreover, HPT was measured using an insulated container and a thermocouple thermometer . In contrast, Briggs et al., (1999) reported inconsistencies on HPT and fat, there was a temperature rise across the pellet die of 3.6°, 2.9°, and 3.2° C on diets amended with high-oil corn + soybean meal (**SBM**) (4.9% fat), corn + expelled SBM (5.6% fat), and high-oil corn + expelled SBM (7.5% fat), respectively. Compared to the Corey et al. (2014) study, the total % of fat in the diet was determined through proximate analysis thus no fat was added at the mixer, mash diets were conditioned at 77° C and HPT was measured using a foam-insulated pail with a stiff thermocouple after the temperature reading reached equilibrium.

In experiment 2, a lower HPT was obtained when the sample was analyzed with the IR gun compared with samples analyzed with the insulated thermos and the Styrofoam bucket in treatments 4 (2% MW, 2% CS), PC (0% MW, 4% CS) ($P \leq 0.01$), and NC (0% MW, 0% CS) (P

≤ 0.05) (Figure 3.3). In agreement with experiment 1, the temperature readings obtained with the IR gun fluctuated at ambient temperature which allowed heat to escape within the pellets, leading to an underestimation of HPT. In the case of water addition at the mixer, Pope and Fahrenholz (2020) reported no statistical significance on ΔT (change in temperature between hot pellet and conditioned mash) with 0, 10, and 20 g/kg of mixer-added water in a swine gestation diet. However, the authors reported statistical differences on ΔT of 6.1°, 3.8°, and 1.5° when diets were conditioned at 80°, 86°, and 92° C, respectively. Compared to the NC diet, the 4% MW is adding enough lubrication to mitigate some of the frictional impact of the die in the PC2 diet. Moreover, the difference between the two PCs diets may be due to the action of steam better penetrating the particles which affected the capture methods. Therefore, temperature readings for these diets are more directly related to the steam than the friction thus the insulated thermos and Styrofoam bucket are better at determining this value while the IR gun only captures the surface of the pellets. Based on the results of these experiments, the repelling relationship of fat + water addition during mixing, conditioning, and post-pelleting processes could play a major role in temperature increase across the pellet mill die other than mixer or post-pellet application.

In both experiments, there were no statistical differences when the HPT was evaluated using the insulated thermos and Styrofoam bucket for both experiments (P ≥ 0.01) (Figure 3.2 and 3.3). In previous studies, insulated containers + thermocouples/digital thermometers appeared to be the most common non-contact method to measure HPT and provide adequate estimations of HPT based on conditioning parameters and diet formulation (Briggs et a., 1999; Salas-Bringas et al., 2007; Corey et al., 2014; Pope and Fahrenholz, 2020; Evans et al., 2021). Salas-Bringas et al. (2007) evaluated the use of IR thermography as a non-invasive and non-

contaminating method to measure HPT in poultry diets. The device consisted of an IR camera and a pellet sampler/collector installed on the pellet mill door facing one of the sides of the rotating die/pellets. The authors concluded that die friction at the beginning of the run (higher temperature profiles) and the additional friction and strains of the rolls and die ring also have an impact on HPT, thus recommended a search for new technologies ideally, in-line. The differences in results among studies could be attributed to the equipment used, manufacturing techniques, type of fat, conditioning temperatures, and conditioner retention time, hence the rise of temperature on pellets at die exit or at die surface due to frictional forces or lubrication require further investigation. Therefore, the objective of experiment 3 was to compare an in-line device that could better estimate "initial" heat rise of pellet temperature the moment pellets are extruded by the die.

Contact method: Temperature profile probe-based tool

The main effects of fat inclusion levels, conditioning temperatures, and die L:D ration on HPT are reported in Table 3.5. A fat inclusion of 4% and a conditioning temperature of 74° C had the lowest HPT compared with diets amended with 1% fat, conditioned at 79° and 85° C ($P \leq 0.01$). Evans et al. (2021) reported lower ΔT in mash diets conditioned at 85° C compared to 74° C supporting the lubricating properties of moisture in the die. In the case of die thickness, HPT increased by 6° C when diets were pelleted with a 10 L:D ratio compared to a 6.5 L:D ratio ($P \leq 0.01$). Despite the beneficial effects of thicker dies on pellet quality (Behnke, 1990; Buchanan et al., 2010; Wamsley and Moritz, 2013), an increase in effective thickness has been reported to have a negative impact on heat-labile ingredients due to a higher residence time (Bayley et al., 1968; Plavnik et al., 1997). In the present study, die retention time was calculated and a die with 6.5 and 10 L:D ratio retained pellets for approximately 1.46 s and 1.94 s,

respectively (Table 3.4). Therefore, pellets were expose for a longer time to the frictional forces of the die and agree with the differences observed in HPT for both L:D ratios.

The interaction effects of fat inclusion levels, conditioning temperatures, and die L:D ration on HPT are reported in Table 3.5. All interactions were statistically significant ($P \leq 0.01$). The highest HPT was observed on diets amended with 1% fat inclusion, pelleted with a 10 L:D ratio die, and steam-conditioned at 85° C. In contrast, HPT decreased at lower conditioning temperatures, thinner die, and increased amount of fat. Treatments with 1% fat, 6.5 L:D, 85° C and with 4% fat, 10 L:D, 79° C generated similar HPT readings but different die specifications and fat content. It can be concluded that a thinner die requires a conditioning temperature of 85° C and 1% fat to reach the same temperature that a thicker die with 4% fat. In summary, there was an interaction because the fat lubricating properties facilitated feed passage through the die while there was a reduced time of contact of the mash with the die-hole walls at lower conditioning temperatures.

Even though the profile probe was able to capture a large number of readings in a short period of time, two of the sensing channels (#3 and #4) were damaged and lost during the pelleting run. Moreover, sensing point #8 (closest to the die) reported higher HPT compared with the other seven sensing points, the temperature never captured a higher temperature than those registered by the conditioner across the 30 min pellet run. However, based on the data logger recordings, the probe seemed to be slowly catching up with a longer time exposure of the probe with the environment of the pelleting chamber. In agreement, Salas-Bringas et al. (2007) reported the one of the disadvantages with contact methods (e.g., thermocouples) is that the conditioned particles agglomerate in the probe surface which results in a slow time response.

Based on the results of this study, the authors suggest a modification of the current device and select a temperature sensor more resistant to aggressiveness of the pellet mill die.

CONCLUSIONS AND APPLICATIONS

1. Based on the results of experiment 1 and 2, the use of the insulated thermos and Styrofoam bucket provide adequate estimations of HPT as non-contact methods in broiler and swine diets. However, the IR gun may not be the appropriate non-contact methodology to measure HPT as it tends to underestimate the frictional heat generated by the pellet mill die.
2. Main and interactive effects were observed for fat inclusion levels, L:D ratios, and conditioning temperatures in broiler diets. Diets amended with 4% fat, pelleted with a 10 L:D die ratio, and steam-conditioned at 85° C resulted in the highest HPT.
3. In experiment 3, a temperature profile-based tool can be used to monitor HPT inside the pelleting chamber without interfering with the pelleting process and increasing costs. Although, the sensing points of the temperature profile probe-based tool located in close proximity to the pellet mill die reported the highest HPT, they were never higher than the conditioning temperatures. Therefore, a more resistant device with a larger sensing surface area may be required to measure HPT more precisely inside the pelleting chamber.

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Table 3.1. Ingredient and nutrient composition of a swine lactation diet used in experiment 1.

Ingredient, %	Lactation
Corn	74.77
Soybean Meal, 48 % Crude Protein	17.50
Poultry Oil	4.00
Monocalcium phosphate, 21% P	1.50
Calcium carbonate	1.08
Sodium chloride	0.51
Liquid Lysine	0.39
Trace mineral premix[1]	0.14
L-Threonine	0.07
Vitamin Premix[2]	0.04
	100.00

Abbreviation: AME_n, apparent metabolizable energy

[1]Mineral premix contained the following concentrations of minerals: Fe (Iron), 7.3%; Zn (Zinc), 7.3%; Mn (Manganese), 2.2%; Cu (Copper), 1.1%; I (Iodine), 198 ppm; and Se (Selenium), 198 ppm.

[2]Donated by DSM Nutritional Products North America. Vitamin premix includes the following quantities per kg of premix: Vitamin A (Vitamin A acetate), 20568783 IU; Vitamin D (cholecalciferol), 2932090 IU; Vitamin E (DL-alpha tocopherol acetate), 117504 IU; menadione (menadione sodium bisulfate complex), 9700 mg; Vitamin B12 (cyanocobalamin), 73 mg; folacin (folic acid), 4409 mg: D-pantothenic acid (calcium pantothenate), 58790 mg; riboflavin (riboflavin), 14690 mg; niacin (niacinamide), 88183 mg; and D-biotin (biotin), 589 mg.

Table 3.2. Ingredient and nutrient composition of a broiler starter diet used in experiment 2.

Ingredient, %	Starter
Corn	58.62
Soybean Meal, 48 % Crude Protein	34.77
Poultry by Product Meal	2.26
Poultry Oil	2.00
Calcium carbonate	0.84
Sodium chloride	0.50
D-L Methionine (99%)[1]	0.24
Trace mineral premix[2]	0.20
Choline Chloride	0.20
Dicalcium phosphate, 18.5% P	0.14
L-Lysine-HCl (78%)	0.08
Vitamin Premix[3]	0.05
Selenium, 0.06%[4]	0.05
Phytase[5]	0.02
L-Threonine	0.02
Xylanase[6]	0.01
	100.00
Calculated analysis, % (unless otherwise noted)	
AMEn, kcal/kg	3,000
Crude Protein	22.95
Digestible Lys	1.29
Calcium	0.85
Available phosphorus	0.43

Abbreviation: AME_n, apparent metabolizable energy

[1]Donated by Evonik Corporation

[2]Mineral premix include per kg of diet: Mn (manganese sulfate), 120 mg; Zn (zinc sulfate), 120 mg; Fe (iron sulfate monohydrate), 80 mg; Cu (tri-basic copper chloride), 10 mg; I (ethylenediamine dihydriodide), 2.5 mg; and Co (cobalt), 1 mg.

[3]Donated by DSM Nutritional Products North America. Vitamin premix includes per kg of diet: Vitamin A (Vitamin A acetate), 6600 IU; Vitamin D (cholecalciferol), 1980 IU; Vitamin E (DL-alpha tocopherol acetate), 33 IU; menadione (menadione sodium bisulfate complex), 2 mg; Vitamin B12 (cyanocobalamin), 0.02 mg; folacin (folic acid), 1.1 mg: D-pantothenic acid (calcium pantothenate), 11 mg; riboflavin (riboflavin), 6.6 mg; niacin (niacinamide), 55 mg; thiamin (thiamin mononitrate), 2 mg; D-biotin (biotin), 0.13 mg; and pyridoxine (pyridoxine hydrochloride), 4 mg.

[4]Selenium premix provided Se at 0.3 mg/kg of feed.

[5]Quantum® Blue 5G (Donated by AB Vista Feed Ingredients, Marlborough, UK) provides per kg of diet: 500 FTU/kg of phytase activity.

[6]Econase XT was added to provide 16,000 BXU/kg of feed.

Table 3.3. Ingredient and nutrient composition of a broiler starter diet used in experiment 3.

Ingredient, %	Starter
Corn	58.95
Soybean Meal, 48 % Crude Protein	31.75
Poultry by Product Meal	5.00
Poultry Oil	1.00
Monocalcium phosphate, 21% P	0.90
Calcium carbonate	0.84
Sodium chloride	0.50
D-L Methionine (99%)[1]	0.30
Trace mineral premix[2]	0.20
Choline Chloride	0.20
L-Lysine-HCl (78%)	0.15
L-Threonine	0.10
Vitamin Premix[3]	0.05
Selenium, 0.06%[4]	0.05
Phytase[5]	0.01
	100.00
Calculated analysis, % (unless otherwise noted)	
AMEn, kcal/kg	3,000
Crude Protein	22.83
Digestible Lys	1.37
Calcium	1.00
Available phosphorus	0.50

Abbreviation: AME_n, apparent metabolizable energy

[1]Donated by Evonik Corporation

[2]Mineral premix include per kg of diet: Mn (manganese sulfate), 120 mg; Zn (zinc sulfate), 120 mg; Fe (iron sulfate monohydrate), 80 mg; Cu (tri-basic copper chloride), 10 mg; I (ethylenediamine dihydriodide), 2.5 mg; and Co (cobalt), 1 mg.

[3]Donated by DSM Nutritional Products North America. Vitamin premix includes per kg of diet: Vitamin A (Vitamin A acetate), 6600 IU; Vitamin D (cholecalciferol), 1980 IU; Vitamin E (DL-alpha tocopherol acetate), 33 IU; menadione (menadione sodium bisulfate complex), 2 mg; Vitamin B12 (cyanocobalamin), 0.02 mg; folacin (folic acid), 1.1 mg: D-pantothenic acid (calcium pantothenate), 11 mg; riboflavin (riboflavin), 6.6 mg; niacin (niacinamide), 55 mg; thiamin (thiamin mononitrate), 2 mg; D-biotin (biotin), 0.13 mg; and pyridoxine (pyridoxine hydrochloride), 4 mg.

[4]Selenium premix provided Se at 0.3 mg/kg of feed.

[5]Quantum® Blue 5G (Donated by AB Vista Feed Ingredients, Marlborough, UK) provides per kg of diet: 500 FTU/kg of phytase activity.

Table 3.4. Die retention time of two pellet mill die length-to-diameter ratio (L:D) in experiment 3[1].

	L:D ratio[2]	
	6.5	10
Internal die surface (cm^2)	121.62	121.62
Number of holes per cm^2	2.33	2.33
Effective volume per hole (cm^3)	0.49	0.66
Material density (g/cm^3)	2.52	2.52
Amount of material in the effective length of the die (g)	349	463
Mass flow rate (g/s)	240	240
Die retention time[3]	1.46	1.94

[1]Experiment 3 constituted a 2 × 2 × 3 factorial arrangement of 2 fat inclusion levels (1% and 4%), 2 die L:D ratios (6.5 and 10), and 3 conditioning temperatures (74°, 79°, and 89° C).
[2]Length-to diameter ratio. Pellet mill die hole diameter: 4.4 mm, die thickness: 29 mm and 45 mm.
[3]Die retention time was calculated for both L:D ratios using the formula proposed by Saensukjaroenphon et al. (2019): *Die retention time (s) = Amount of material in the effective length of the die (g) / Mass flow rate (g / s)*
The internal die surface area, number of holes per cm^2, effective volume per hole, material density (g/in^3) were multiplied to calculate the amount of material in the effective length of the die (g).

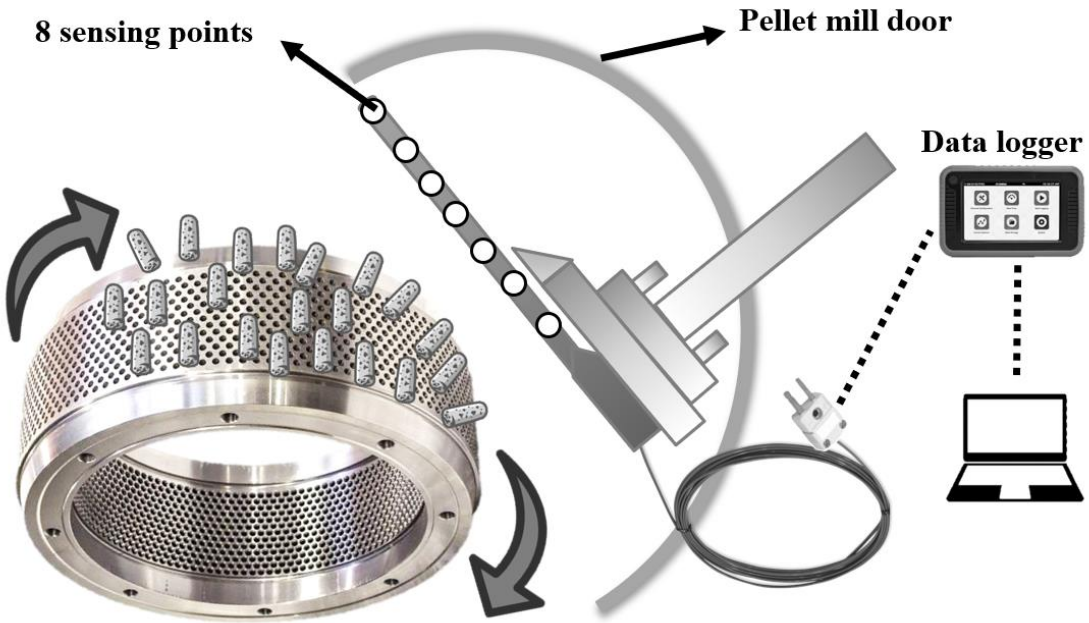

Figure 3.1. Diagram of a custom-made temperature profile probe attach to a pellet mill knife. A custom-made 8-point profile probe type J thermocouple (OSK2K4334 OmegaTM profile probes, Norwalk, CT) was mounted to a pellet mill knife (California Pellet Mill Co., Crawfordsville, IN). The probe was mainly composed of a 1.59 × 8.89 cm transitioning fitting attached to a 19.05 cm (length) stainless steel thermocouple with a grounded junction. Each sensing point had a distance of 1.27 cm and a tolerance of continuous temperatures up to 260° C. Moreover, the transitioning fitting of the probe was attached to a 101.6-inch PFA lead wire terminating in 8 miniature connectors (one for each sensing point). The mini flat blad male terminating miniature connectors with integral cable clamp caps were removed and adapted to fit a data logger. Readings were then recorded by the data logger (Model OM-CP-OCTPRO OmegaTM portable data acquisition logger, Norwalk, CT) while running the pellet mill and exported to a computer for further analysis.

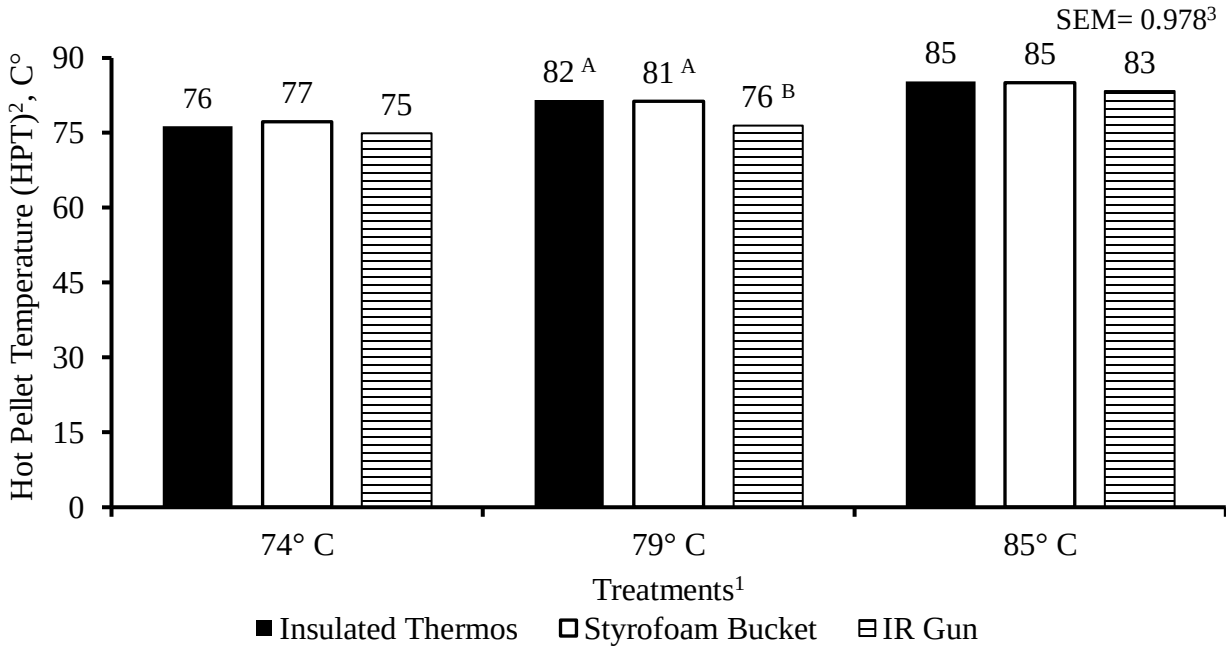

Figure 3.2. Hot pellet temperature of a swine lactation diet varying in conditioning temperatures, experiment 1. [A, B]Means within a column with different superscripts differ significantly ($P \leq 0.01$). [1]Diets were conditioned at three conditioning temperatures (74° C, 79° C, and 85° C). [2]A total of 91 HPT readings were collected using three non-contact methodologies: 1) Insulated thermos (0.13 gal), 2) Styrofoam bucket (5 gal), and 3) Infrared (IR) gun. Pellets were collected at die exit, placed in the container (insulated thermos and styrofoam bucket), and temperature was measure by inserting a thermometer. The highest temperature reading registered by the thermometer was considered for further analysis. The handheld IR gun was pointed directly to pellets at die exit. [3]SEM = Standard error of the means for non-contact methodologies effect (n = 3).

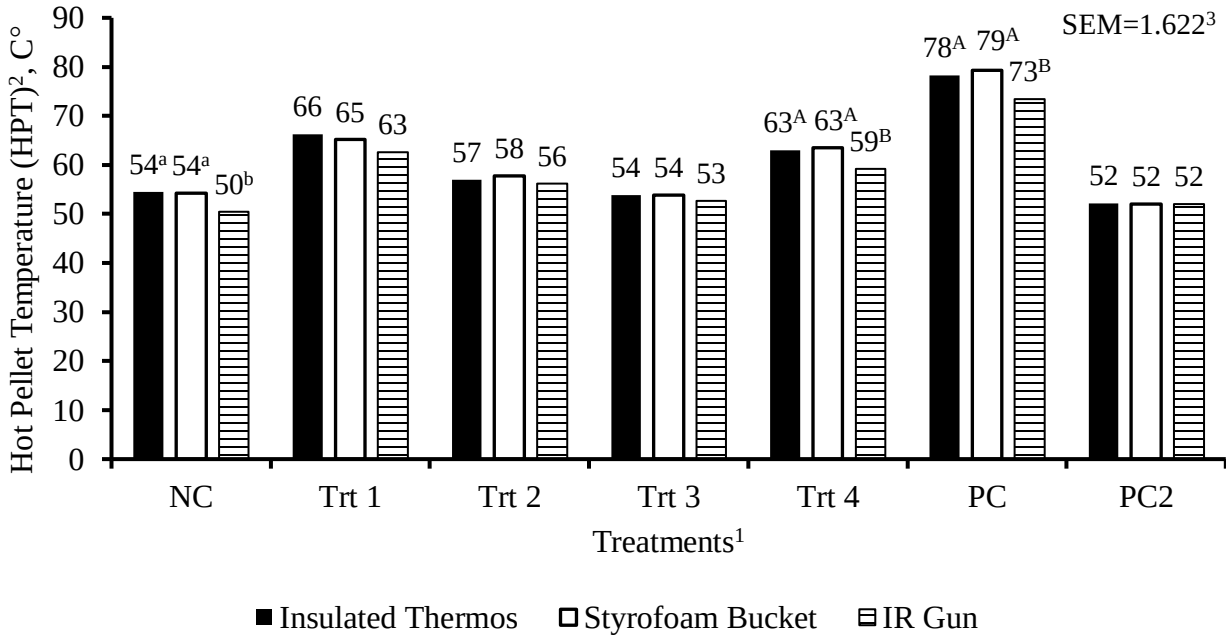

Figure 3.3. Hot pellet temperature of broiler starter diets varying in experiment 2. [a, b]Means within a column with different superscripts differ significantly ($P \leq 0.05$). [A, B]Means within a column with different superscripts differ significantly ($P \leq 0.01$). [1]A broiler starter diet with 7 treatments, varying moisture contents from mixer added water (MW) (0, 1, and 2%) and conditioner added steam (CS) (0, 2, 4%): 0% MW, 2% CS (Trt 1), 1% MW, 1% CS (Trt 2), 2% MW, 0 % CS (Trt3), and 2% MW, 2% CS (Trt 4). A mash diet not subjected to MW nor CS was dry pelleted to serve as negative control (NC). A treatment subjected to 0% MW, but 4% CS served as a positive control (PC) to replicate the standard pelleting conditions in the industry. A treatment subjected to 4% MW and 0% CS served as a second positive control (PC2). [2]A total of 189 HPT readings were collected using three non-contact methodologies: 1) Insulated thermos (0.13 gal), 2) Styrofoam bucket (5 gal), and 3) Infrared (IR) gun. Pellets were collected at die exit, placed in the container (insulated thermos and styrofoam bucket), and temperature was measure by inserting a thermometer. The highest temperature reading registered by the thermometer was considered for further analysis. The handheld IR gun was pointed directly to pellets at die exit. [3]SEM = Standard error of the means for non-contact methodologies effect (n = 3).

Table 3.5. Main and interaction effects of fat level, pellet mill die length-to-diameter ratio (L:D), and conditioning temperature on hot pellet temperature (HPT) as determined by a temperature profile probe in experiment 3.

Fat level[2]	L:D ratio	Conditioning	n	HPT, C°
Main effects				
1%			330	74.64^{A}
4%			330	72.30^{B}
P-value				<.0001
SEM[1]				0.02
	6.5		330	70.50^{B}
	10		330	76.44^{A}
	P-value			<.0001
	SEM			0.02
		74°	220	69.60^{C}
		79°	220	73.96^{B}
		85°	220	76.85^{A}
		P-value		<.0001
		SEM		0.03
Interaction effects				
1%	6.5	74°	55	68.09^{I}
1%	6.5	79°	55	74.88^{E}
1%	6.5	85°	55	77.85^{C}
4%	6.5	74°	55	64.15^{K}
4%	6.5	79°	55	67.68^{J}
4%	6.5	85°	55	70.35^{H}
1%	10	74°	55	71.53^{G}
1%	10	79°	55	75.58^{D}
1%	10	85°	55	79.91^{A}
4%	10	74°	55	74.63^{F}
4%	10	79°	55	77.69^{C}
4%	10	85°	55	79.31^{B}
P-value				<.0001
SEM				0.05

$^{A, B}$Means within a column with different superscripts differ significantly ($P \leq 0.01$).

[1]SEM =Standard error of the mean for n=330 readings for each main effect of fat level, n=330 readings for each main effect of pellet mill die L:D, n=220 readings for each main effect of conditioning temperature, and n=55 readings for each interaction of fat level, pellet mill die L:D, and conditioning temperature.

[2]Poultry oil was added at 1% or 4%.

OVERALL CONCLUSIONS

Mixer and pellet mill equipment could be identified as alternatives to improve feed manufacturing efficiency in modern feed manufacturing facilities, thus it is important to acknowledge the improvements that have been made with regards to mix uniformity and feed thermostability. However, the majority of mixing and pelleting research focused solely on traditional methodologies to evaluate feed quality parameters. The goal of this research was to explore available technologies and implement novel methodologies to monitor nutrient homogeneity in finished feeds and heat rise within pellets and how they affect broiler growth and frictional heat during pelleting.

The data in Chapter 1 indicated that mix uniformity had no impact on broiler growth performance (BW, BWG, FI, FCR, and mortality) and individual BW uniformity during the starter, grower, and finisher periods. Although it was expected that chicks during the starter period would require increased mix uniformity (mix time), broilers were able to consume diets with a total mix time of 0.5 min without a negative impact on growth. A moderate reduction in total mix time of broiler diets during the grower and finisher periods could be an alternative to improve labor hours in a commercial setting. Sodium chloride is commonly used to test mixer performance, nevertheless the coefficient of variation (CV) observed by titration methods were not below the accepted standard ($\leq 10\%$) of the industry. This was related to on-site analysis vs. more precise analytical tests used for other markers commonly incorporated in broiler diets such as trace minerals and amino acids. However, some of these markers selected are cost-prohibitive thus may not be feasible for high throughput facilities. Moreover, not all markers are added at the same level or measured using the same analytical techniques which can also affect the interpretation of mix uniformity.

The data in Chapter 2 further expanded on marker selection, mix time, mixer fill, and methodology used to evaluate mixer performance. An In-line FT- near infrared (NIR) using fiber optics and connected to a probe was welded into the surge conveyor of the NCSU feed mill and made data collection simple and efficient. In agreement with previous research, an increase in total mix time resulted in a more uniform mix. In addition, filling the mixer to half or full rated capacity did not have an impact on mix uniformity. Although the protein values from the proximate analysis generated by the In-line NIR were ≤ 10%, there were no differences at both mix times. It seems that the Microtracers followed a similar trend to estimate the degree of variation in mixed feed compared to sodium chloride and the In-line NIR. When broiler diets are amended with L-Lysine HCL and choline chloride, chloride ion titration methods may not be the most effective. Even though the mixer CVs with the In-line Nir were not as expected, the incorporation of "biases" to protein values could be an alternative to improve the responses observed. The visual representations provided by the spectra readings demonstrated differences between the runs and a larger distribution was observed on diets with a total mix time of 0.5 min at 908 and 1815 kg batch sizes. The reference spectra used (4.5 min and 1815 kg) allowed a higher number of spectra readings thus allowing to observe a distinct comparison with the other runs. In the case of a shorter mix time, some samples were highlighted to observe a gap on max CI at two different batch sizes. Therefore, it can be concluded that the use of the In-line NIR could be used to estimate mix uniformity and provides visual representations of the "behavioral pattern" of finished feed through conveying systems in real time.

The data in Chapter 3 indicated that insulated containers provide a better estimate of heat rise within pellets compared to non-invasive methods such as infrared devices. The goal of this experiment was to compare the current available non-contact methodologies used by feed

manufacturers to estimate the additional heat generated by the pellet mill. While the impact of conditioning temperatures on thermos-sensitive nutrients have been extensively reviewed, it is a challenge to demonstrate the "actual" impact of die specifications during pelleting. Therefore, the welding of a temperature profile probe proves to be a magnifying glass as to what is happening to pellets as they are extruded by the die. There is an agreement that the development of contact methods is difficult due to the aggressive environment within the pelleting chamber. This was confirmed with the loss of multiple sensing points during the experimental runs. Additionally, the slow readings of the probe did not allow the collection of temperatures higher than those of the conditioner. However, despite the slower responses of the probe, the sensing point located in closest proximity to the pellet die had the highest temperatures. A future solution for these slower responses is to install a sensor that is more resistant to steamy and intrusive environments. The location of the pellet mill knife (base of the probe) is accurate for data collection and is in close contact with the pellets. These data indicated that from a die specification and formulation standpoint, L:D ratio and inclusion level of fat seem to have the largest impact on HPT, respectively.